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VASCULAR DIFFERENTIATION IN THE PEAR ROOT[1]

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INTRODUCTION

THE PRESENT PAPER deals with the development of the root of *Pyrus communis* L., with special attention to the vascular tissues. Though considerable information is available on root structure of herbaceous plants (Hayward, 1938; Esau, 1940),[3] only one rather complete account of tissue differentiation in a root of a woody species appears to exist in modern botanical literature (Hayward and Long, 1942). The need for such accounts in the teaching of plant anatomy, especially in agricultural institutions, is obvious.

The present problem was selected also because of the writer's interest in the differentiation of the phloem tissue in different organs of seed plants. Since many studies have been made on the phloem of roots of herbaceous plants (review by Esau, 1943), it seemed timely to add some data on the ontogeny of this tissue in a woody root.

MATERIALS AND METHODS

The root material used in preparing the permanent slides and the photomicrographs was obtained from trees grown in a culture solution by the Plant Nutrition division at Berkeley. The material was killed in a formalin-acetic-alcohol fixing fluid and imbedded in paraffin after ordinary dehydration and clearing in mixtures of ethyl alcohol and xylene.

The roots grown in the culture solution were compared with soil-grown roots from young trees 5 to 6 inches high grown from seeds of the Winter Nelis variety. The latter roots were examined in free-hand sections. No fundamental differences were found between those grown

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[3] See "Literature Cited" for complete data on citations mentioned in the text by author and date of publication.

in culture solution and those grown in soil.[4] All roots examined in the primary state were lateral, since the apices of the seedling taproots were not available. The apical meristem was studied only in the culture-solution material.

PRIMARY ORGANIZATION OF THE VASCULAR CYLINDER

The apex of the root shows the common separation into the primordial vascular cylinder (or stele), the immature cortex, and the rootcap (plate 1, *B*). In the apical-meristem region, however, only the stele is set off from the other regions with its own initials. The cortex, the epidermis, and the rootcap have a common origin.

The stelar initials form a uniseriate layer at the apex of the stele (*si* in plate 10). By periclinal divisions these initials give rise to the innermost part of the stele. Nearer the periphery of the initial layer the divisions are intermediate between the periclinal and the anticlinal, and they are entirely anticlinal in the outermost initial cells. The anticlinal divisions add cells to the pericycle. In plate 10 the pericycle (*p*) is evident as a layer of elongated cells immediately inside the white line on the sides of the stele. In this figure the pericycle can be followed as a uniseriate layer directly into the initial region. In other words, the pericycle of this root tip is individualized immediately behind the apical initials. The peripheral derivatives of the stelar initials may, however, undergo a periclinal division before the pericycle is delimited. In any case, the pericycle is histogenetically a part of the stele, and early becomes individualized.

The initial region giving rise to the cortex, the epidermis, and the rootcap, is composed of several layers of cells (*ci* in plate 10, about five layers of cells below the white line in the center of the figure). On the sides of this region anticlinal divisions contribute cells to the cortex. Acropetally the initial cells produce the core of the rootcap by periclinal divisions. The immediate products of these divisions also divide mainly periclinally, so that the youngest part of the rootcap core shows rather orderly-arranged longitudinal files of meristematic cells merging with the initials of the root apex (plate 10). The orderly files of cells remain evident also after the maturation of the rootcap core (plates 1, *B*, and 10). The peripheral portion of the rootcap is produced by periclinal and oblique divisions from the outermost lateral derivatives of the apical meristem. These derivatives are here interpreted as cortical cells and not as epidermis because the latter is set off from the cortex and the rootcap some distance from the apical meristem, after the divisions

[4] Dr. A. S. Foster furnished the killed and imbedded material of roots grown in culture solution, while Dr. L. D. Davis supplied the soil-grown material.

producing the rootcap cease. The peripheral rootcap cells are also aligned in longitudinal files at the source of their origin (plate 10), but this arrangement is somewhat disturbed during the further development of the cells (plate 1, *B*).

Judging by Schüepp's (1926) discussion of root-meristem organization, the pear-root apices described in the present paper belong to the type in which "the entire outer part of the cortex contributes toward the formation of the rootcap" and the stelar initials are independent of those producing the cortex, the epidermis, and the rootcap (Schüepp, 1926, p. 70, type III B). According to Schüepp, certain Rosaceae belong to this group.

The depth of the initial region and the subsequent periclinal divisions in the cortical meristem determine the final thickness of the cortex. Although some doubling up of the longitudinal cell layers occurs throughout the youngest region of the cortex, the addition of new cells through periclinal divisions in its innermost layer is more conspicuous. Plates 10 and 2, *A*, show the result of this meristematic activity. A succession of periclinal divisions in the innermost cortical layer form several rows of narrow cells, densely cytoplasmic. Farther away from the pericycle the cells are larger, their protoplasts less dense. Some anticlinal divisions also occur as the root increases in circumference. After completion of the periclinal divisions the innermost layer of cortical cells undergoes a differentiation as an endodermis (plate 3, *B*, *en*). Eventually the anticlinal divisions and the change in shape of the endodermal cells obscure their close histogenetic relation to the adjacent cortical layer (fig. 1; plate 3, *B*). Since the last periclinal divisions in the inner cortex may not pass all around the stele, the limit between the endodermis and the adjacent cortical layer may appear somewhat disorderly (fig. 1; plate 3, *B*).

The meristematic stele (the procambium) of the root shows a cytologic differentiation immediately behind the apical initials. The central region quickly develops conspicuous vacuoles, and the cells enlarge (plate 10). Comparatively few longitudinal divisions occur here. When the peripheral region is formed by the apical initials these, as was mentioned previously, divide obliquely; and the immediate derivatives undergo periclinal (longitudinal) divisions. Because of these divisions the periphery retains a meristematic appearance somewhat longer than the center of the stele (plate 10). Although nearest the apex the entire periphery of the stele is densely meristematic, some 200 microns higher the peripheral region becomes lobed through the increased vacuolation of certain portions of it. The densely cytoplasmic portion of the procambium becomes broken up into strands, whereas the more highly

vacuolated part assumes, in transverse sections, the appearance of a star (plate 2). Eventually the strands differentiate into the primary phloem, the vacuolated part of the stele into the primary xylem. Thus the xylem and phloem regions become delimited some 200 to 300 microns from the apex, and the metaxylem region is vacuolated before the protoxylem region. The longitudinal divisions that occur in the

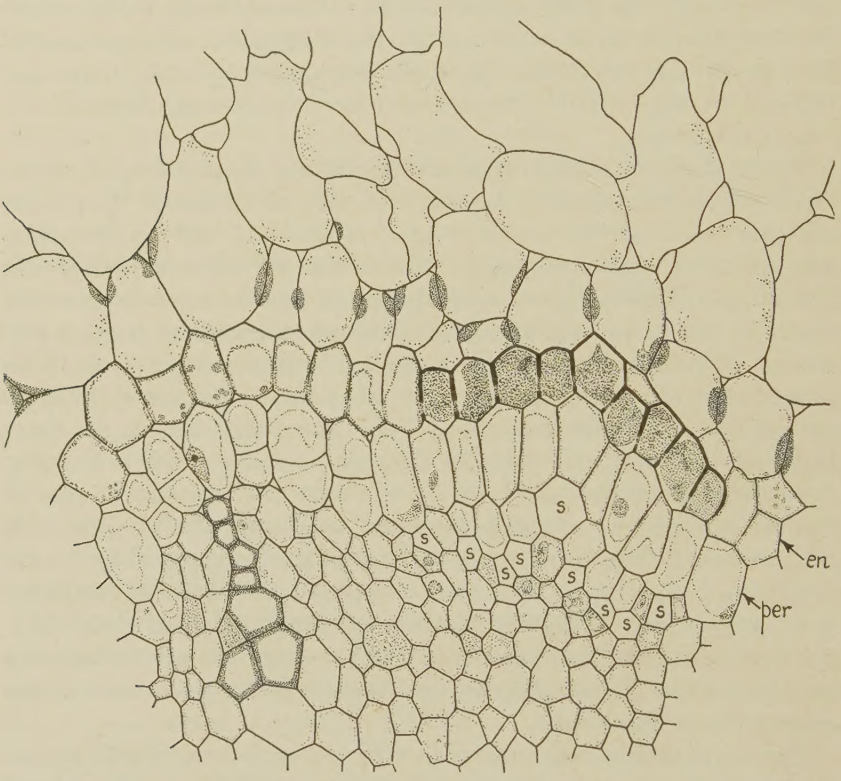

Fig. 1.—Transverse section through portion of root, illustrating the characteristics of the endodermis. The drawing was made from the same section as in plate 3, *B* (area within the rectangle). Details are: *en*, endodermis; *per*, pericycle; *s*, sieve tube. The faint areas in the endodermal walls indicate sections of the Casparian strips. Above the endodermis is the cortical layer with localized wall thickenings. (× 621.)

peripheral region of the stele last longest on the inner margins of the procambium strands that give rise to the phloem. Later the vascular cambium is initiated in this position (plate 4, *B*). The young pericyclic cells are as densely cytoplasmic as the future phloem cells, but larger (plate 2, *A*).

Densely staining inclusions, usually interpreted in the literature as being tannic in nature, appear in the xylem region, the endodermis, and

the cortex (plates 1, *B*, and 2, *A*). The early distribution of the tannic inclusions in the endodermis and in the adjacent cortical layers shows a peculiar relation to the stelar regions. Plate 2, *B*, for example, shows tannin throughout the endodermis and in certain groups of the adjacent cortical cells—groups located next to the regions of the stele that would later have differentiated into the protoxylem. In older stages of root development the tannic inclusions become dispersed throughout the cells instead of remaining confined to the peripheral cytoplasmic layer. This phenomenon is first noticeable in the parts of the endodermis next to the protophloem poles (plate 3, *B*, and fig. 1). Later all endodermal cells and scattered cells within the other root regions stain uniformly densely throughout the protoplasts, apparently because of the dispersed tannic inclusions (plates 3, *A*; 4; 5; 6, *B*; 7).

At levels located about 600 to 700 microns from the apical meristem the first sieve-tube elements differentiate, one in each phloem strand (plate 2, *B*). They mature at unequal levels at the different poles. In the root shown in plate 2, *B*, the section where the first of the five sieve tubes matured was 80 microns nearer the apex than the section where the fifth sieve tube was fully differentiated. The first sieve tubes appear next to the pericycle and are not associated with any cells that could be interpreted as companion cells (plate 6, *A*, sieve tube, *s*, in the center of the figure). Although, as was pointed out before, the pericycle is early individualized, periclinal divisions may occur in the outermost layer of the stele during the organization of the phloem, and a sieve-tube element may differentiate as a sister cell of a pericyclic cell (plate 6, *A*, sieve tube, *s*, in the center of the figure).

These first differentiated phloem elements merit the designation as sieve-tube elements because they have sieve plates on their more or less inclined terminal walls, lack nuclei, and show lightly stained mature protoplasts—all characteristics common to the protophloem sieve-tube elements of angiosperms. (See review by Esau, 1939.) The sieve-tube elements are about 70 microns long immediately upon maturation.

Additional sieve-tube elements differentiate at each pole laterally from the first sieve tubes. In plate 6, *A*, the first sieve tube of one of the phloem strands is in the center of the figure; the second appears to the right. The additional sieve tubes have companion cells but, like the first ones, appear next to the pericycle. When the xylem begins to differentiate, about two sieve tubes occur at each phloem pole. Then still more sieve-tube elements differentiate, some next to the pericycle, others in deeper layers of the procambium strand—that is, centripetally from the first sieve tubes (fig. 1). All these subsequent sieve tubes have companion cells.

Shortly before the xylem begins to differentiate, the entire stele becomes highly vacuolated even in the phloem and the pericycle regions (plate 6, *A*). The sieve tubes are therefore much less conspicuous in the more mature regions (plate 3, *B*) than nearer the apex, where they stand out as cells with lightly stained contents among the densely cytoplasmic procambial cells (plate 2, *B*).

Some 5 mm from the apex the deposition of the secondary walls is initiated in the first xylem elements. These cells differentiate next to the pericycle at the protoxylem poles that alternate with the protophloem poles. The relative position of the early xylem and phloem elements may be judged from figure 1 and plate 3, *B*, showing two views of the same section taken 2 cm from the apex of the root. The number of the protoxylem poles and, correspondingly, that of the protophloem poles varies, four (plates 3, *A*, and 5, *B*), five (plates 2, *B*; 4; and 5 *A*), and six (plate 3, *B*) having been observed in roots of larger, and two in roots of smaller diameters.

The distances from the apex to the first mature xylem and phloem elements were determined by the use of roots grown in culture solution. These distances are not necessarily comparable with those that would occur in roots grown in a different environment, but they vary also in roots grown under similar conditions (Esau, 1941). The differentiation of the sieve tubes in advance of the xylem elements appears, however, to be a usual phenomenon in roots. (See review by Esau, 1943.)

The first xylem elements have very narrow diameters (fig. 1) and in the culture-solution material show scalariform secondary thickenings. One or two elements at each pole are of this nature, the subsequent ones being reticulate and pitted. The later xylem elements have a greater diameter than the first (fig. 1). In soil-grown material the first elements were also scalariform or transitional between spiral and scalariform. Differences in the types of the secondary walls of the protoxylem can be expected in roots grown under different environmental conditions. As has been well established experimentally, the nature of the secondary walls is related to the degree of stretching—caused by the elongation of the entire organ—to which the elements are subjected during their differentiation and thereafter (Smith and Kersten, 1942). Whether the first xylem elements in the pear root are tracheids or vessels has not been ascertained; for convenience they are here called the *xylem elements* or *tracheary elements* (Foster, 1942, p. 80).

In the section shown in plate 3, *B*, about five to nine sieve tubes occurred at each protophloem pole, and about three to six mature tracheary elements. (In the section shown in figure 1 the two lowermost xylem elements were still immature.) At this stage of development the

endodermis shows Casparian strips. These structures, which are very inconspicuous, appear to be imbedded in the primary wall without forming a thickening on its surface. In sections stained with fast green and safranin they are evident as red wall areas contrasting with the green stain in the rest of the walls. As usual they occur on the radial and transverse walls near the inner tangential walls. The Casparian strips are somewhat more conspicuous in the endodermal cells located at the protophloem poles, probably because in these positions the endodermal walls are somewhat thicker than next to the protoxylem poles (fig. 1).

The cortical layer immediately outside the endodermis is characterized by prominent wall thickenings that resemble those of collenchyma cells (fig. 1; plates 3, *B;* 4; 5; and 7, *A*). These thickenings are not very bright in polarized light, are not lignified, and appear as bands in longitudinal views. Most of them occur on the radial walls, though some are located on parts of walls adjacent to the intercellular spaces (fig. 1; plates 4 and 7, *A*). Occasionally the thickenings also occur in the cortical layer second from the endodermis (fig. 1 and plate 7, *A*). Similar modifications of the inner cortical cells have been mentioned in the literature. Russow (1875, p. 72–73) referred to the similarly thickened cortical layer outside the endodermis as the *exodermis* and commented that the transverse sections of the thick walls resembled the Greek letter Phi. He reported such an exodermis in the roots of the Pomaceae, specifically mentioning *Pyrus,* and in certain other families of dicotyledons and gymnosperms. Guttenberg (1940, p. 121–22) calls this layer the *inner cortical sheath* and records its presence in the Rosaceae.

At the stage of root development illustrated in plate 3, *B,* and figure 1, the pericyclic cells show pronounced radial elongation and divide periclinally next to the protoxylem poles. As viewed in longitudinal sections the pericyclic cells appear short, in sharp contrast to the long cells of the adjacent vascular tissues. Plate 8, *A,* illustrates this difference in a root somewhat older than the one shown in plate 3, *B.*

On the basis of our present information regarding the stages in the differentiation of the primary vascular tissues (review by Esau, 1943), the terms *protophloem* and *protoxylem elements* are here applied to the phloem and xylem cells which mature in advance of the other vascular elements in the root and which by their position mark the pattern of differentiation followed by the primary vascular tissues. As is usual in roots, the phloem following the protophloem in time of appearance (that is, the metaphloem) and the subsequent primary xylem (the metaxylem) differentiate centripetally from the protophloem and protoxylem poles respectively. The phloem also spreads laterally from its

points of initiation, so that the protoxylem strands eventually appear as narrow strips of tissue crowded between the broad phloem strands (plate 4, *A*).

The demarcation between the protophloem and the metaphloem, and between the protoxylem and metaxylem is usually drawn somewhat arbitrarily (Esau, 1943). The first sieve-tube element at each pole in the pear root is the largest in diameter among the primary sieve tubes and lacks companion cells (plate 3, *B*, and fig. 1). These are probably rather common characteristics of the first sieve tubes of dicotyledonous roots (Esau, 1935, 1940, 1941). In time of appearance the first sieve tubes in the pear root are less sharply set off from the following sieve tubes than they are in tobacco-root tips studied by the present writer (Esau, 1941). In the pear root, as was mentioned earlier, one or two additional sieve-tube elements having rather narrow diameters and associated with companion cells differentiate at each pole before the first xylem elements begin to show a deposition of secondary walls.

Since there is no sure basis for delimiting the different parts of the primary vascular tissues (review by Esau, 1943), the first three or four elements at each pole (elements early crushed because of subsequent growth changes in the root) are here classified as protophloem and protoxylem elements. Conceivably, the distinctness with which the first vascular elements are set off from the subsequent ones in time of appearance is determined largely by the degree of elongation of the roots. Judging by the nature of the secondary walls of the protoxylem in the pear root (scalariform, rather than annular or spiral) and by the small amount of distortion that these walls show in sections of root with secondary growth, the roots used in this study must have been elongating only slightly, if at all, after the protoxylem matured. The lateral pressure of the adjacent living cells seems in this material to have been the principal cause of distortion of the protoxylem elements. Plate 5, *B*, indicates the encroachment of the adjacent cells upon the protoxylem, particularly at the protoxylem pole in the lower part of the figure. The crushing and the obliteration of the protophloem sieve tubes and their companion cells, if these are present, are rather conspicuous (plate 4, *B*, and 7).

By the definitions given above the sieve-tube elements in plate 6, *A*, are protophloem cells. The mature intact sieve-tube elements in plate 7, *A*, are metaphloem cells. The two immature sieve tubes at the lower left in plate 7, *A*, are the first secondary sieve tubes in this bundle. Certain cells of parenchymatous appearance in plate 7, *A*, are phloem-parenchyma cells, whereas others are much elongated elements and eventually differentiate as fibers. Plate 7, *B*, illustrates the early stage

in secondary-wall formation in the fibers of the protophloem, whereas plate 8, *B*, shows one of these fibers on the outer limit of the phloem in longitudinal view. Later, fibers differentiate in the metaphloem also. The primary-phloem fibers together with the fibers of the earliest secondary phloem form, in old roots, compact strands on the outer periphery of the vascular cylinder (plate 1, *A*, *fb*). The sieve tubes and companion cells are all obliterated in this region, while the parenchyma cells are much dilated, especially in the tangential direction. The parenchyma cells contain inclusions such as starch, tannin, and crystals; some of them become sclerified as stone cells. As the present writer has frequently emphasized (Esau, 1938, 1939, 1943), fibers that appear on the outer periphery of the vascular cylinder in stems commonly arise in the phloem. Lloyd (1911, p. 94) has given good evidence that the peripheral fibers of the root stele in *Parthenium* are phloem fibers.

The metaxylem comes to occupy the entire center of the stele (plates 1, *A*; 5; 6, *B*; and 8, *A*). Vessels, tracheids, and xylem parenchyma, all prominently pitted, occur in this region. Though the metaxylem is defined before the protoxylem in the meristematic stele, it matures rather slowly, so that its secondary-wall formation is not terminated before cambial activity sets in (plate 4, *B*).

SECONDARY GROWTH IN THE ROOT

As has been pointed out, the procambial divisions last longest on the inner margins of the phloem bundles. During the differentiation of the protophloem and protoxylem, the procambial cells in this position enlarge somewhat and vacuolate so that they merge imperceptibly with the immature phloem and xylem cells (plates 6, *A*, and 4, *A*; fig. 1). After the final delimitation (but not maturation) of the primary regions in the stele (plate 4, *A*) the divisions on the inner margins of the phloem bundles are resumed and now result in radial series of narrow cells (plate 4, *B*). These are divisions initiating the secondary growth of the vascular tissues.

As shown in plate 4, *B*, the first cambium occurs in isolated curved strips on the inner sides of the phloem bundles. This meristem, after producing some secondary xylem elements, becomes united into a continuous layer between the xylem and the phloem by the meristematic activity of the pericyclic cells located outside the protoxylem poles. Plates 4, *B*, and 5 show how markedly this early production of secondary xylem changes the outline of the cambium region in transverse sections. First it appears in the form of curved arcs, bulging toward the center of the root (plate 4, *B*); then the arcs are straightened out (plate 5, *A*). After this position is attained, periclinal divisions in the pericycle out-

side the protoxylem also form some cambium, and thereby this meristem becomes a continuous, more or less cylindrical layer of tissue around the entire circumference of the xylem. In plate 5, *B*, this stage had almost been reached. As previously indicated, the pericyclic cells divide periclinally at a very early stage in the primary development of the root (fig. 1). These divisions probably prepare the formation of the vascular cambium in this position.

In common with the vascular meristem of arborescent dicotyledons, the cambium of the pear root is composed of fusiform and ray initials. The pericyclic cells outside the protoxylem give rise to ray initials, so that vascular rays radiate from the protoxylem poles through the secondary vascular tissues (plate 1, *A*). In agreement with Barghoorn's (1940) observations on ray formation in roots, the rays formed at protoxylem poles in the pear root are the first multiseriate rays in the secondary xylem. The cambium arising in the procambium inside the primary-phloem strands also produces rays, but the earliest formed in this position are uniseriate.

Plate 1, *A*, shows a pear-root section with considerable secondary growth of the first season. The primary xylem, a five-pointed star, is imbedded in the secondary xylem. In the latter the wide pores (representing transverse views of vessels) and the rays are the conspicuous structural features detectable at this magnification. Plate 9, *B*, shows a section of the secondary xylem from plate 1, *A* (area delimited by a rectangle), in greater detail. Four rays are visible in this section, the one to the left being a multiseriate ray that arose in the pericycle outside the protoxylem. (Compare with plate 1, *A*.) The rays are parenchymatous and contain starch grains and tannin. In the longitudinal system the cells having the widest diameters (plate 9, *B*) are vessels. Some of the narrower cells are also tracheary elements; others are fibers and xylem-parenchyma cells. Starch grains and tannin occur in the latter.

The major part of the tissue located outside the cambium in plate 1, *A*, is phloem (*ph*). The rest is pericycle and periderm. The multiseriate and uniseriate parenchymatous phloem rays that are continuous with the xylem rays divide the secondary phloem into blocks of tissue composed of sieve tubes, companion cells, phloem parenchyma, and some fibers. A portion of the secondary phloem from plate 1, *A* (area delimited by a rectangle) is depicted at high magnification in plate 9, *A*. A multiseriate ray occurs to the left. In the lowermost part of this figure appears the cambium. Then follows the functioning part of the phloem with mature sieve tubes (*s*). Farther away from the cambium the sieve tubes and the companion cells are partly crushed among the enlarged parenchyma cells. This is the phloem part which, according to a common

concept, is no longer concerned with longitudinal conduction. The enlargement of the phloem parenchyma cells becomes particularly conspicuous towards the periphery of the stem, where a considerable tangential dilation occurs in all living cells of the phloem and pericycle.

The outer limits of the phloem may be determined by the position of the fibers which, as was described earlier, arise in the phloem, the earliest ones being in the protophloem (plate 7, *B*). The pericycle gives rise to the cork cambium. The formation of this meristem is preceded by an increase in thickness of the pericycle. As previously mentioned, the earliest tangential divisions in this region occur outside the protoxylem (fig. 1, plate 3, *B*). Later, such divisions spread all around the periphery of the stele (plates 5, *A*, and 7, *B*) and are repeated several times, so that the pericycle shows a marked increase in thickness (plates 5, *B*, and 8, *B*). In plate 5, *B*, the excessive width of the pericycle to the right of the stele results from growth phenomena associated with the development of branch roots. The latter are surrounded at their bases by collars of tissue resulting from a proliferation of pericyclic cells. In sectional views these collars resemble lenticels, provided the branch roots associated with them do not appear in the same view (plate 1, *A*, below). True lenticels have not been observed in the present material.

During the increase in the circumference of the stele through the cambial activity and the proliferation of the pericycle, the cortex together with the endodermis is crushed and sloughed off. Plate 3, *A*, shows the first splitting of the cortex. No cortex occurs in the section in plate 1, *A*.

The new pericycle cells are aligned in rather orderly radial rows (plates 5, *B*, and 8, *B*). The outer cells become tangentially stretched and radially compressed as the stele increases in circumference. After undergoing suberization they serve as a protective layer before the cork cambium and cork are formed in one of the deeper layers in the pericycle. In the material used in this study most of the sections showed only the first-formed periderm. Occasional roots showed isolated strips of cork cambium within the secondary phloem.

SUMMARY

The apical meristem of the root shows two sets of initials. One set, one layer deep, produces the stele with the pericycle; the other set, several layers deep, gives rise to the cortex, the epidermis, and the central core of the rootcap. The peripheral portion of the rootcap arises from the youngest cortical cells.

Within the stele the pericycle is individualized almost directly behind the apical initials. The phloem and the xylem regions are clearly

delimited before any vascular elements differentiate, the phloem region being composed of small, densely cytoplasmic cells, the xylem region of larger and more highly vacuolated cells.

The first protophloem sieve tubes mature in advance of the first protoxylem elements. The protophloem and the protoxylem appear next to the pericycle and alternate with each other. The number of the protophloem and protoxylem poles varies in roots of different diameters.

The differentiation of the metaphloem and metaxylem proceeds in the usual centripetal manner from the protophloem and protoxylem poles respectively.

The endodermis is a uniseriate layer with Casparian strips. The cortical layer next to the endodermis has localized thickenings on its walls.

The cambium arises in the manner characteristic of roots. It first appears on the inner side of the phloem bundles, then becomes continuous across the pericycle cells located outside the protoxylem. The secondary vascular tissues show the common characteristics of these tissues in woody dicotyledonous roots.

The pericycle, which is at first uniseriate, becomes multiseriate by tangential divisions. The outermost cells resulting from these divisions become cork cells, and beneath them arises a cork cambium. The cortex with its endodermis is sloughed off in connection with the secondary activity in the stele.

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PLATES

Plate 1.—*A*, Transverse section of a root with considerable secondary growth. Details are: *c*, cambium; *fb*, fibers; *ph*, phloem; *r*, rays. The rectangles delimit the areas of secondary phloem and secondary xylem depicted at high magnification in plate 9. The small arrowheads in the center indicate the five protoxylem poles. *B*, Longitudinal section of a root tip showing the apical meristem and the regions immediately derived from it. (*A*, × 50; *B*, × 90.)

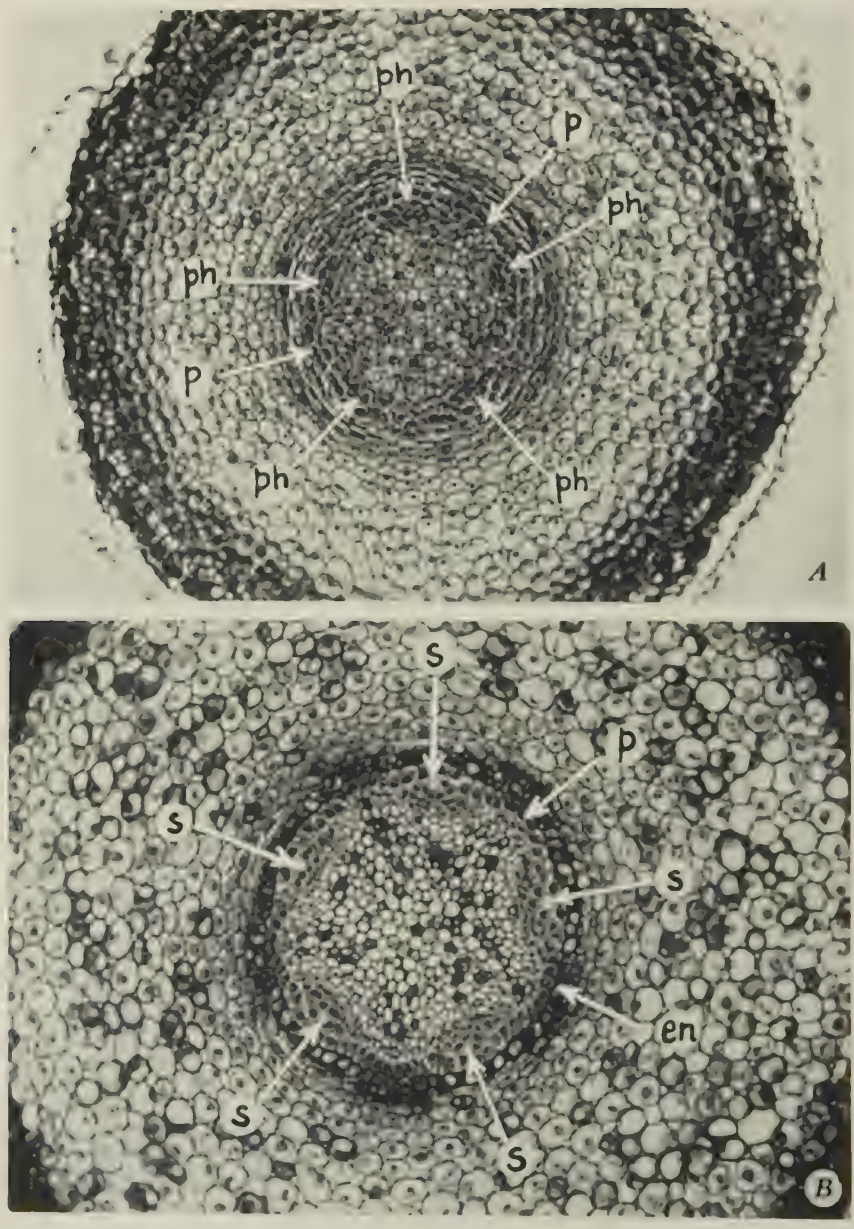

Plate 2.—Transverse sections of a root tip taken 280 microns (*A*) and 750 microns (*B*) from the apical meristem. Details are: *en*, endodermis; *p*, pericycle; *ph*, protophloem pole; *s*, sieve tube. (Both × 180.)

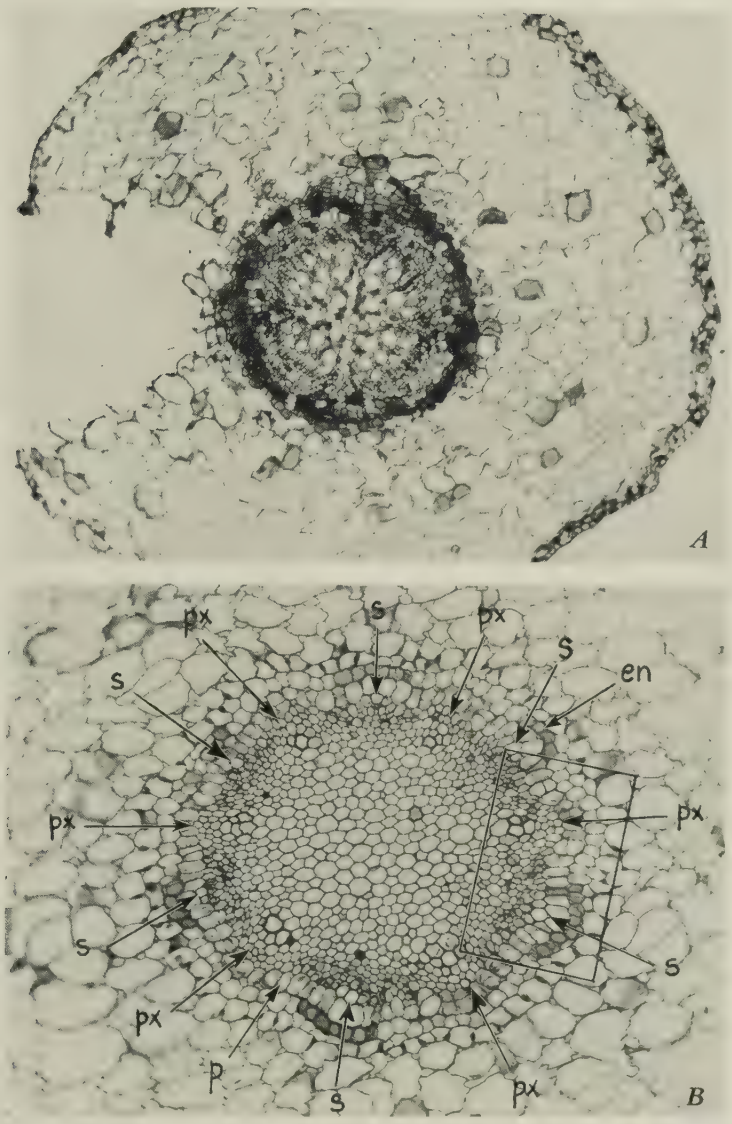

Plate 3.—*A*, Transverse section of root, showing splitting of cortex. The stage of development of the vascular tissues is comparable to that in plate 5, *B*. *B*, Transverse section of root taken about 2 cm from the apex. It illustrates an early stage of phloem and xylem differentiation. Details are: *en*, endodermis; *p*, pericycle; *px*, protoxylem; *s*, sieve tube. The rectangle delimits the area depicted at higher magnification in figure 1. (*A*, × 90; *B*, × 180.)

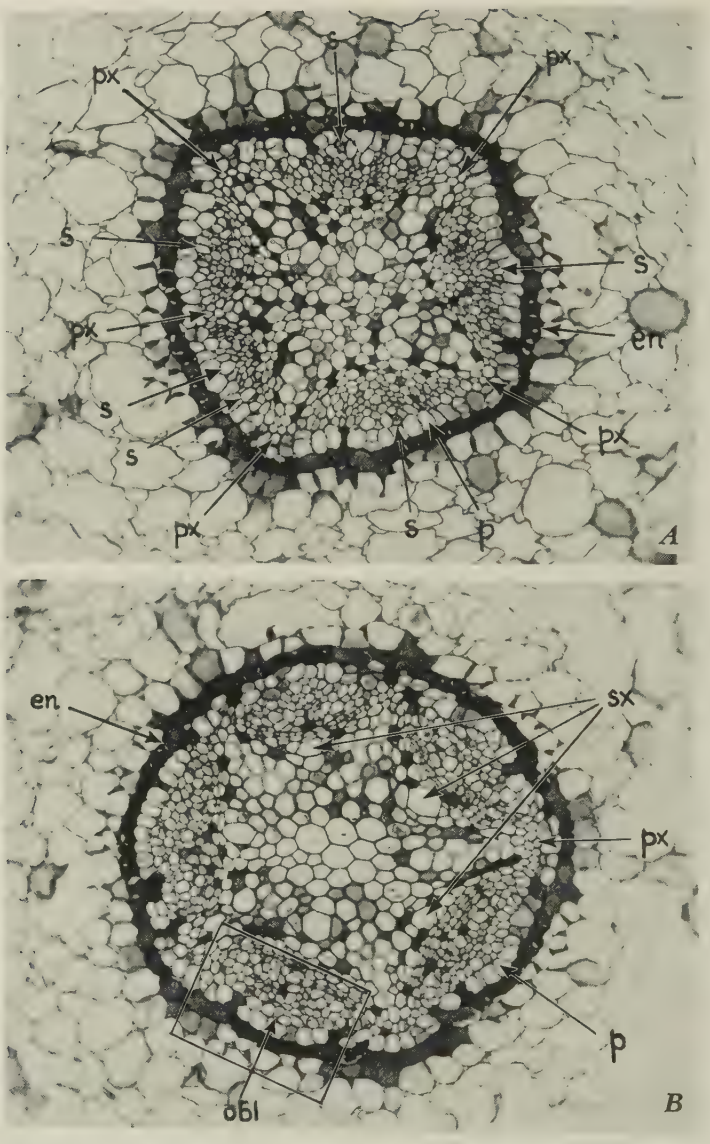

Plate 4.—Transverse sections taken about 4 cm (*A*) and 5 cm (*B*) from the apex of the root. *A* illustrates the stage just before the beginning of cambial activity. In *B* the first secondary xylem elements are present. Details are: *en*, endodermis; *obl*, obliteration of sieve tube; *p*, pericycle; *px*, protoxylem; *s*, sieve tube; *sx*, secondary xylem. The rectangle in *B* delimits the area shown at higher magnification in plate 7, *A*. (Both × 180.)

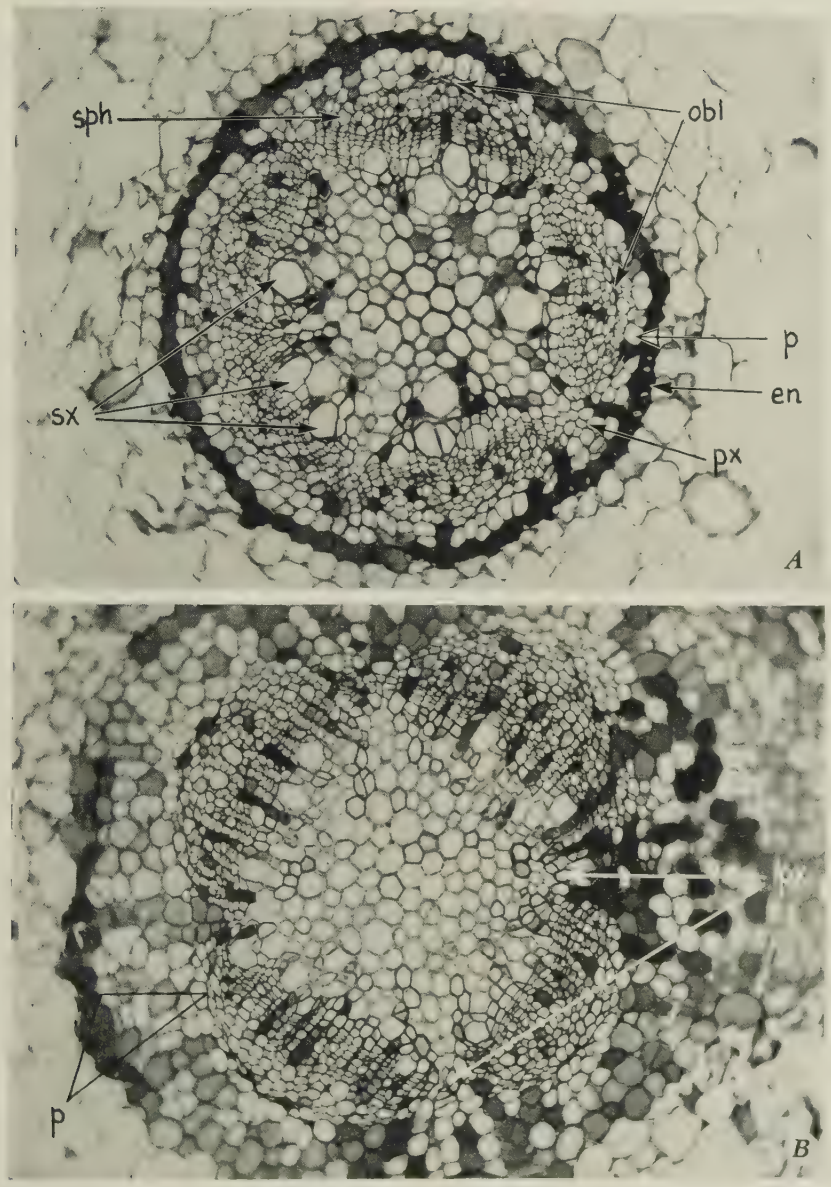

Plate 5.—Transverse sections taken about 7 cm (*A*) and 9 cm (*B*) from the apex of the root. *A* illustrates the stage just before the splitting of the cortex. In *B* the cortex was split. Five protoxylem poles occur in *A*, four in *B*. Details are: *en*, endodermis; *obl*, obliteration of sieve tube; *p*, pericycle; *px*, protoxylem; *sph*, secondary phloem; *sx*, secondary xylem. (Both × 180.)

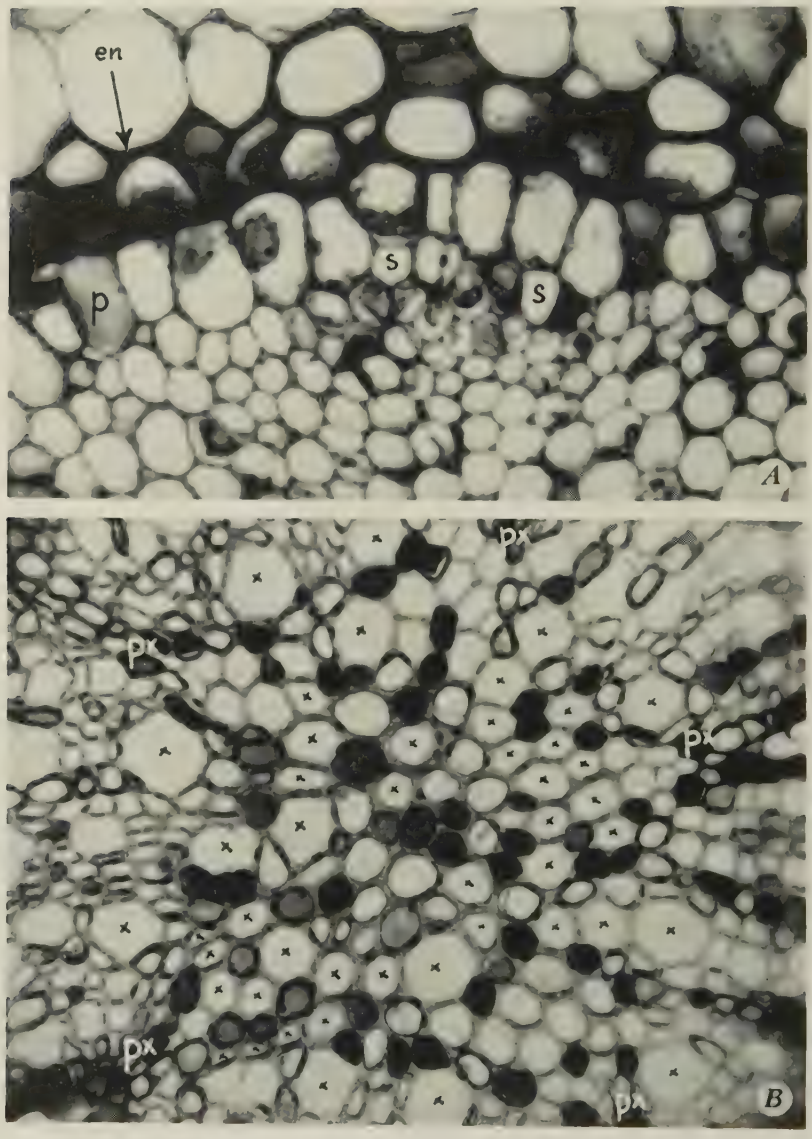

Plate 6.—*A*, Transverse section through a protophloem pole with two sieve tubes (*s*) taken 1,720 microns from the apical meristem. *B*, High-power view of the primary-xylem region from a section somewhat older than that shown in plate 5, *B*. Details are as follows: *en*, endodermis; *p*, pericycle; *px*, protoxylem; *s*, sieve tube. In *B* the tracheary elements have been indicated by small crosses. (*A*, × 810; *B*, × 360.)

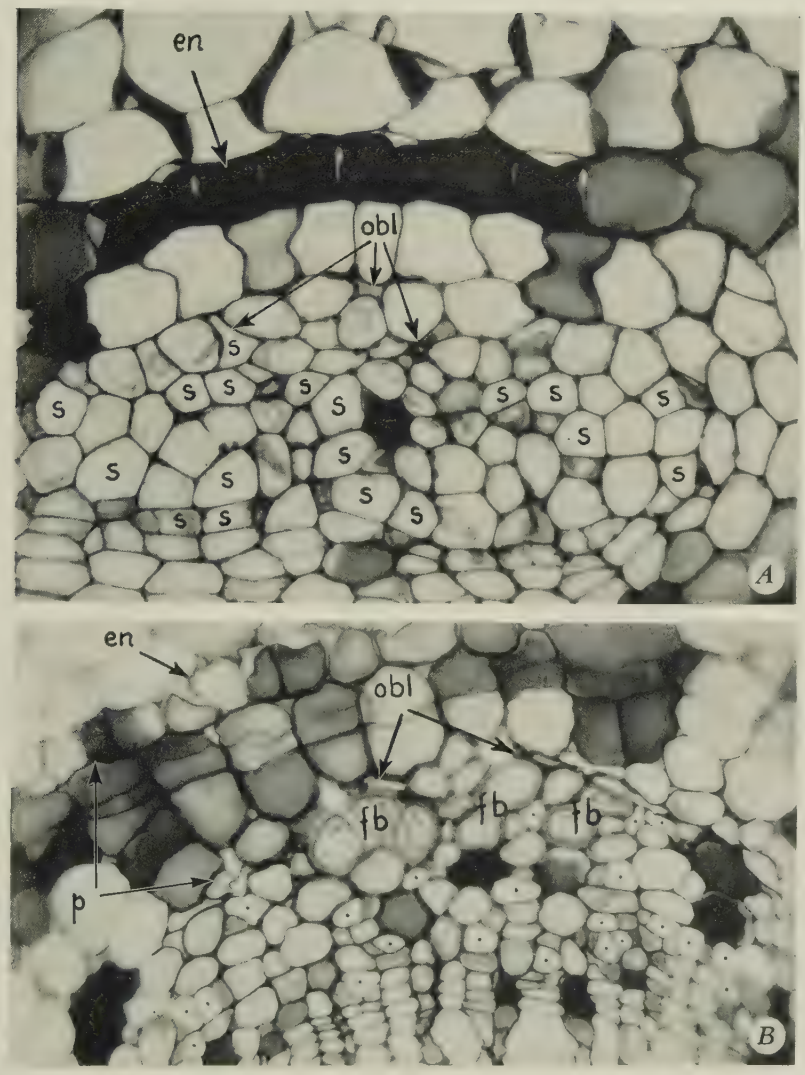

Plate 7.—*A*, High-power view of portion of transverse section figured in plate 4, *B* (area within the rectangle). It shows the primary phloem, the endodermis, and the inner cortical sheath above the endodermis. *B*, High-power view of portion of transverse section figured in plate 3, *A*. It shows the primary phloem during the early stage of fiber development and the first secondary phloem near the cambium. Details are: *en*, endodermis; *fb*, fiber; *obl*, obliteration of sieve tubes; *p*, pericycle; *s*, sieve tube. The sieve tubes in *B* are marked by dots. (*A*, × 810; *B*, × 540.)

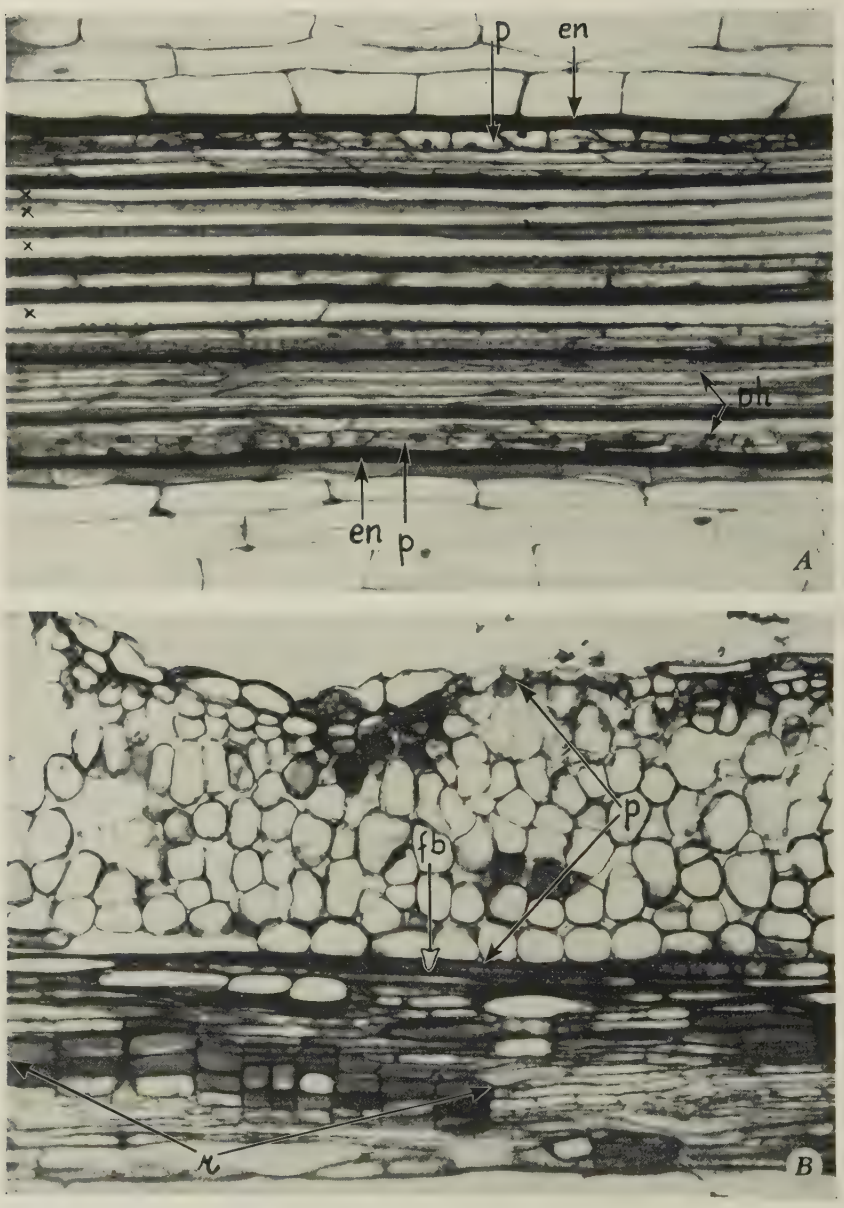

Plate 8.—*A*, Radial longitudinal section of root at the end of primary growth as in transverse view in plate 5, *A*. The cells marked with small crosses are pitted tracheary elements. *B*, Radial longitudinal section of a root sampled after the proliferation of the pericycle as in transverse section in plate 5, *B*. Details are: *en*, endodermis; *fb*, fiber in the phloem; *p*, pericycle; *ph*, phloem; *r*, ray. (Both × 180.)

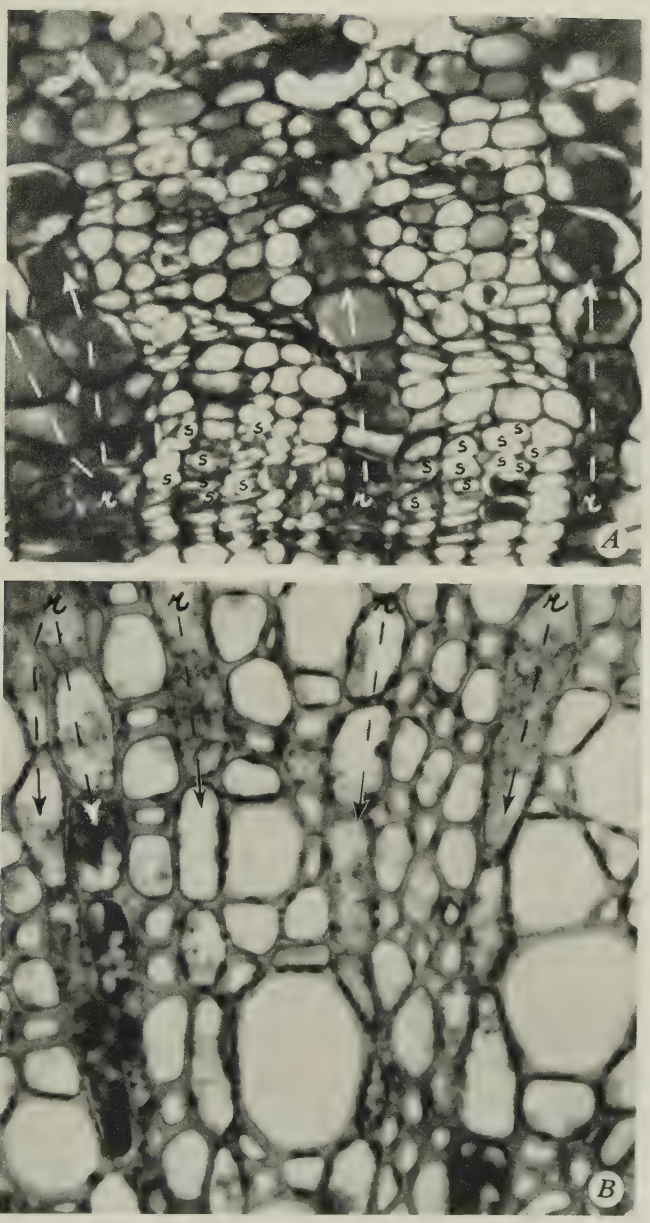

Plate 9.—Portions of transverse sections of secondary phloem (*A*) and secondary xylem (*B*) from the same section as in plate 1, *A* (areas delimited by rectangles). The letter *s* in *A* indicates the sieve tubes in the mature phloem. Above this region is the old phloem with partly crushed sieve tubes and prominent parenchyma cells. The letter *r* indicates the rays in both figures. (Both × 360.)

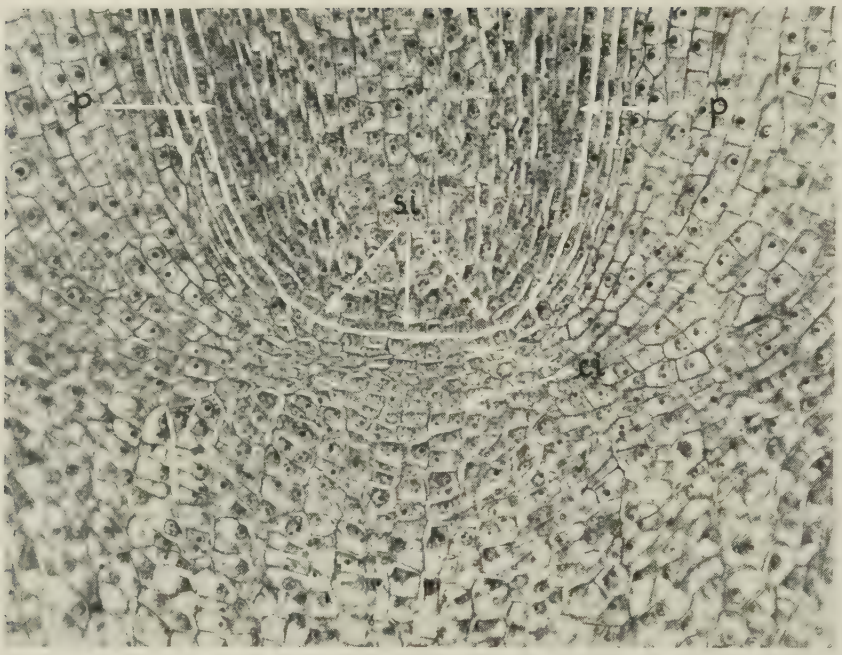

Plate 10.—Median longitudinal section of root tip through the apical-meristem region. The limits of the stele are indicated by a white line. Details are: *ci*, cortical initials; *p*, pericycle; *si*, stelar initials. (× 300.)

ONTOGENY OF THE VASCULAR BUNDLE IN ZEA MAYS

KATHERINE ESAU

ONTOGENY OF THE VASCULAR BUNDLE IN ZEA MAYS[1]

KATHERINE ESAU[2]

CONTINUING THE STUDIES on the anatomy of crop plants, with special emphasis on vascular differentiation (Esau, 1936*a*, 1938, 1940, 1941),[3] the writer has selected *Zea Mays* L. as a representative of the monocotyledons. Though the vascular system of this plant has often been studied (Strasburger, 1891, p. 329–63; Hayward, 1938, chapter 5; Sharman, 1942), the ontogeny of the vascular strand merits further detailed investigation in view of the many unsolved problems of vascular differentiation in the monocotyledons. (See review by Esau, 1943.) The present paper concerns, first, the developmental relation between the vascular tissues and the bundle sheath—a structure characteristic of the vascular bundles in the Gramineae. Second, it attempts to elucidate the nature of the meristem producing the vascular bundles. Literature gives conflicting answers to the question whether this meristem—some or all of it—should be interpreted as *procambium* or *cambium*. (See review by Esau, 1943.)

The vigorous vegetative side shoots used as material for slides were obtained from the plants of the Golden Cross Bantam variety, growing in an open field. In preparing the permanent slides a common paraffin method (Esau, 1941) was followed. Free-hand sections were employed for examining the gross structural features.

THE MORPHOLOGY OF THE VASCULAR BUNDLE

For clarity the structure of the vascular system and of the mature vascular bundle will be considered before the ontogenetic details. Since the recent works on this subject (Hayward, 1938; Sharman, 1942) have not cited Strasburger's (1891, p. 329–63) thorough treatment of the morphology of the vascular tissues in *Zea Mays*, his description is here reviewed in the light of the present observations and the findings of other investigators.

The Vascular System.—In common with the other monocotyledons, *Zea* has numerous "parallel" vascular bundles in the leaf blade and the leaf sheath. (Since the bundles converge and fuse at the tip of the leaf, they are not truly parallel.) Large bundles alternate with small. Within the stem the prolongations of the leaf strands, the *leaf traces*, appear as

[1] Received for publication October 20, 1942.

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[3] See "Literature Cited" for complete data on citations, mentioned in the text by author and date of publication.

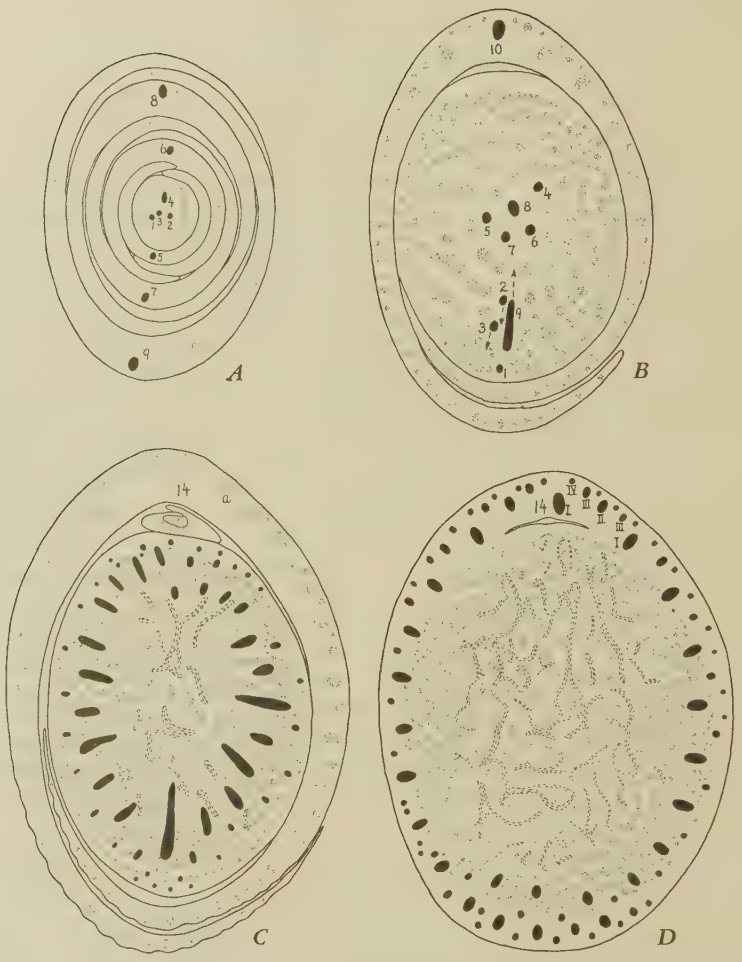

Figure 1.—Diagrams of transverse sections through a shoot taken the following number of microns below the apex: *A*, 450; *B*, 1,030; *C*, 2,690; *D*, 3,320. One or more leaves ensheathe the stem in *A* to *C*. In *A* and *B* the median leaf bundles or traces are shown in black. Traces 1 to 3 appear in their median positions within the stem in *A*, but are near the periphery in *B*. The arrows in connection with the traces 2 and 3 in *B* indicate that these two traces occurred still nearer the periphery at lower levels of the stem. In *C* the traces of leaf 13 are shown in black. The large traces, which at lower levels occur near the center of the stem, have at level *C* a somewhat oblique transverse course. In *D* the traces of leaf 14 are shown in black. The traces of leaf 13 in *C* were cut below the insertion of leaf 13, whereas the traces of leaf 14 in *D* appear at the level where leaf 14 is united with the stem. The small curved bundles indicated by broken lines in *C* and *D* are the horizontal traces leading to the axillary buds and adventitious roots. At *a* in *C* appears a transverse connection between two longitudinal strands in leaf 14. The Roman numerals in *D* indicate the order of appearance of the bundles accompanied by these numbers. (*A* and *B*, × 20; *C* and *D*, × 10.)

scattered vascular bundles (fig. 1, *B*). The different traces of one leaf occur at different depths within the stem. If the traces of a given leaf are studied in successive sections, downward from the node at which this leaf is attached to the stem, their course is as follows: Within the node the large bundles bend inward, whereas the small ones remain near the periphery of the stem (fig. 1, *C*). The median bundle bends so strongly that it approaches the center of the stem. In figure 1, *A*, the median traces 1, 2, and 3 appear in their innermost positions; trace 4 is approaching such a position. In figure 1, *B*, the median traces 4 to 8 occur around the center of the axis. The other large bundles come to occupy intermediate positions between the peripheral and the central. With slight alterations in their positions the traces extend downward within the stem through several internodes; then the larger traces become clearly reoriented to a peripheral position. Figure 1, *B*, shows the median trace of leaf 1 in a peripheral position, whereas traces 2 and 3 are approaching this position. Usually the median trace appears in its peripheral position on the side of the stem opposite the median part of the leaf to which this trace belongs. In this respect traces 1 and 3 in figure 1, *B*, deviate from the ordinary condition. The reorientation of the large traces is accompanied by a basipetal diminution in size, so that in their peripheral prolongations these traces are as small as the leaf traces whose longitudinal course is entirely peripheral.

The vascular system of *Zea* closely corresponds with that of the so-called "palm-type," whose features Haberlandt (1914, p. 383) sums up. Sharman (1942, p. 259) opposes this comparison because the large bundles lateral to the median "do not approach the center of the stem, but maintain a vertical course." Figure 1, *C*, shows that the large lateral traces, as well as the median, have a horizontal course at the node and approach the center of the axis, though finally the laterals occupy a less central position than the median strand.

The traces of a given leaf remain discrete through variable lengths of the stem. Strasburger (1891, p. 351–52) found the larger traces to be free of connections through longer distances than the smaller bundles. He followed the median traces through about six internodes. Sharman (1942) and the present writer distinguished median strands within eight or more internodes in the youngest shoot parts. Traces 1 and 2, for example, are still discrete in the ninth internode in figure 1, *B*. The smallest peripheral bundles may fuse with others at the first node or may continue free to the next lower node (Strasburger, 1891).

Certain horizontal bundles occur in the nodal plates and in the peripheral portion of the internodal base (fig. 1, *D*). In plate 10 these bundles are cut mostly transversely and appear as small groups of

dense cells among the large longitudinal traces. According to Strasburger (1891, p. 346–47), the horizontal bundles of the internode rise from the node below and connect with the axillary buds and the adventitious roots. Sharman (1942) places all the horizontal bundles in the base of the internode, interpreting them as prolongations of the youngest peripheral traces formed after these traces reach the base of the internode in their basipetal course of development. Judging by the position of the leaf bases and the horizontal bundles, the section in plate 10 does not substantiate Sharman's statement that horizontal bundles are not formed within the node. In addition, figure 1, *C*, shows some transverse bundles at the same level where the traces of leaf 13 have a horizontal course within the node of this leaf.

The Vascular Bundles of the Leaf Sheath, the Internode, and the Node.—The vascular strands vary in structure in the different parts of their course. There are also differences between bundles of different size. Plate 9, *B*, depicts a large bundle from a leaf sheath. In structure it resembles the central bundles of the internode. (Compare with plate 25 in Artschwager, 1925.) It is the best-known type of *Zea* bundle. The xylem and the phloem are arranged collaterally and, when mature, enclose no meristem between them. The tracheary elements of the protoxylem (annular and spiral vessels), which mature before the stem or the leaf elongates, are disintegrated in the mature bundles; in their place appears a large intercellular space, the *protoxylem lacuna* (plate 9, *B*, *l*). The lacuna is surrounded by xylem parenchyma. Frequently an annular or spiral vessel appears in the median position of the bundle next to the lacuna (plate 9, *B*). Since this vessel matures after the elongation of the stem or sheath and is not destroyed by this growth, it may be regarded as the first metaxylem element. On both sides of the bundle (plate 9, *B*) occur two other large metaxylem vessels, usually with pitted or reticulate-pitted secondary walls. The small-celled tissue between the two lateral metaxylem vessels (in contact with them) is a mixture of xylem parenchyma and narrow tracheary elements. The latter are small vessels (Strasburger, 1891, p. 330; Cheadle, 1942), varying in number; some of them usually touch the large vessels (plate 9, *B*). Like the conducting elements, the parenchyma of the metaxylem has lignified walls and prominent elongated pits.

The phloem is composed of rather large sieve tubes and small companion cells in a more or less orderly pattern. The phloem shown in plate 9, *A* and *B*, is metaphloem. The protophloem is crushed in mature bundles (*obl* in plate 9). The last procambial cells between the xylem and the phloem differentiate as parenchyma, which separates the two conducting tissues from each other. This separation is not necessarily

complete. Phloem cells (companion cells and sieve tubes) frequently are in contact with the lignified xylem parenchyma. (In plate 9, *B*, the sieve tube at *st* and its companion cell lie next to a xylem-parenchyma cell.)

The large bundles are enclosed in a bundle sheath having lignified walls. The sheath cells are compactly arranged, with no intercellular spaces among them or between them and the vascular cells. At the xylem and the phloem poles of the bundle the sheath is two to several layers thick, the cells being sclerenchymatous, long, and tapering. On the flanks of the bundles the sheath cells are shaped like parenchyma cells; they have rounded pits, in contrast to the slitlike pits of the prosenchymatous sheath cells. Often the sheath is uniseriate on the flanks of the bundle (plate 9, *B*); but it may be thicker. As far as could be ascertained, the sheath cells have protoplasts in mature state, in agreement with Strasburger's (1891) description.

The sheath cells are in contact with the xylem parenchyma and frequently also with the two lateral metaxylem vessels (plate 9, *B*). According to Strasburger (1891), no pits occur between the vessels and the sheath cells, although these structures are very prominent between the vessels and the xylem parenchyma cells. The sheath cells touch the crushed protophloem, but are separated from the metaphloem by a layer of parenchyma (plate 9, *B*). According to Strasburger (1891), these parenchyma cells may show lignification in the walls adjacent to the sheath.

The small peripheral bundles of the stem and the small strands of the leaf sheath have less vascular tissues and smaller bundle sheaths than the large bundles. Since the smallest bundles mature after the organs containing them have ceased to elongate, they show little effect of stretching. No protoxylem lacuna is present, or the protoxylem and the protophloem are entirely lacking.

The central or near-central bundles that traverse the nodes vertically also show little effect of elongation in the nodal region, in that their protoxylem is not converted into a lacuna. Fusions of bundles are common in this region. Traces having a horizontal course in the node (fig. 1, *C*, the large traces of leaf 13) show an arrangement of tissues different from that in the longitudinal bundles: the xylem tends to surround the phloem (amphivasal bundles). The nodal anatomy is further complicated by anastomoses, fusions, and the connections with the horizontal system already mentioned (fig. 1, *D*).

The Bundles of the Intercalary-Meristem Zone.—Zea, in common with the other Gramineae, has the so-called "intercalary-meristem" regions at the base of each leaf sheath and each internode of the stem.

It is commonly stated in the literature that these meristems cause the internodes and the leaf sheaths to elongate for some time after being laid down by the apical meristem (Troll, 1935, p. 109). As Haberlandt (1914, p. 182) implies, fundamentally the intercalary elongation of the shoots in the Gramineae is comparable with that of the dicotyledonous stems. Haberlandt also points out that "there are comparatively few Phanerogams in which cell-formation and -extension are strictly confined to the apical region of the axis and the youngest internodes." In the Gramineae and the Cyperaceae, however, the intercalary growth is very striking, since it appears to be rather clearly separated from the apical growth and occurs within a comparatively short period of vegetative growth. According to Haberlandt (1914, p. 183) and others, the intercalary regions remain "permanently meristematic"; that is, after the shoot has reached its mature length through the activity of the apical and intercalary meristems, the bases of the leaves and of the internodes can elongate further because of the special characteristics of the intercalary zones.

The present observations on the manner of growth of the shoot apex in *Zea* agree essentially with Sharman's (1942). In the youngest portion of the shoot the internodes as such do not exist; they develop through cell division at the base of the leaf-insertion disks. The insertions of the two superposed leaves are thereby separated from each other. In other words, the nodes are removed from each other by intercalary growth. The closeness of origin of two superposed nodes is emphasized by the common origin of the axillary bud of the lower leaf with the base of the next higher leaf. The axillary bud becomes separated from the upper leaf by the interpolation of the internode. The bud, then, according to Sharman, is associated with the internode of the leaf above and not with the leaf in whose axil it appears. Rösler (1928) has previously given the same interpretation of the origin of internodes and axillary buds in wheat. Similarly Evans and Grover (1940) concluded that the bud of a grass develops at the base of a "phytomer" (the unit of structure of the shoot composed of an internode, the leaf at its upper end, and the bud at its lower end) in the axil of the leaf that crowns the "phytomer" next below.

As Sharman has shown, the internode grows by orderly transverse divisions giving rise to longitudinal files of cells. Plate 10 illustrates this phenomenon. The longitudinal section depicted in this plate passed through two nodes (*n*) and one internode (*in*) between the bases of two leaves (*lb*), which were approximately the sixteenth and seventeenth from the apex. The nodes are characterized by the complexity of their vascular systems, but the young internode is composed of longitudinal

files of parenchyma (rib meristem) and of vertical strands of vascular tissue. This internode is in a state of division throughout its length. Later, the meristematic activity becomes confined to the base of the internode (Sharman, 1942). The leaf elongates similarly by intercalary growth, during which the cell divisions are gradually localized in the intercalary-meristem zone of the leaf sheath.

Whether represented by the young internode (or leaf sheath) or by a restricted region in the elongated internode (or leaf sheath), the intercalary "meristem" is a partly differentiated tissue region. Its degree of differentiation varies during the different stages of development of the organ containing it. As the internodes elongate, the dividing parenchyma cells of these regions show increasing vacuolation, and the vascular bundles differentiate through them. The vascular strands passing through the growing internode in plate 10 had mature protoxylem and protophloem. After the shoot parts have elongated, the metaphloem and metaxylem mature. Most parts of the plant become structurally rigid: the last vessels develop pitted walls, the bundle sheath is sclerified, and the hypodermal sclerenchyma forms its thick lignified walls. The final elaboration of the intercalary zones occurs, however, in relation to the retention of meristematic potentialities in these zones. The vascular bundles show a small amount of lignified tissue; and the vessels are of the annular and spiral types, these vessel types being directly continuous with pitted elements above and below. (This observation was reported by Strasburger, 1891, and is confirmed in the present study.) The bundles are not encased in a sheath of lignified cells, but are accompanied by a collenchymatous tissue in the form of massive sheaths or bundle caps. No lignified hypodermal sclerenchyma is formed.

Artschwager (1925) describes similar modifications of bundles in the intercalary-meristem regions in the sugar cane and gives further details on the variations in the structure of a grass bundle in the different parts of the shoot.

The Leaf-Blade Bundles.—The vascular bundles in the flat part of the leaf blade can be divided roughly into three groups according to size and structure. The largest (fig. 2, *E*) have a lignified sheath confluent with the hypodermal sclerenchyma. The sheath cells on the flanks of the bundle are shaped like elongated parenchyma cells and contain abundant large chloroplasts. In figure 2, *E*, the plastids are indicated by stippled circles. The uniseriate sclerenchymatous sheath layers on the xylem and phloem ends of the bundle (confluent with the hypodermal sclerenchyma) are free of chloroplasts. The bundle in figure 2, *E*, has two large lateral metaxylem vessels. Below these is an annular protoxylem vessel, which appears like a space because it is much extended

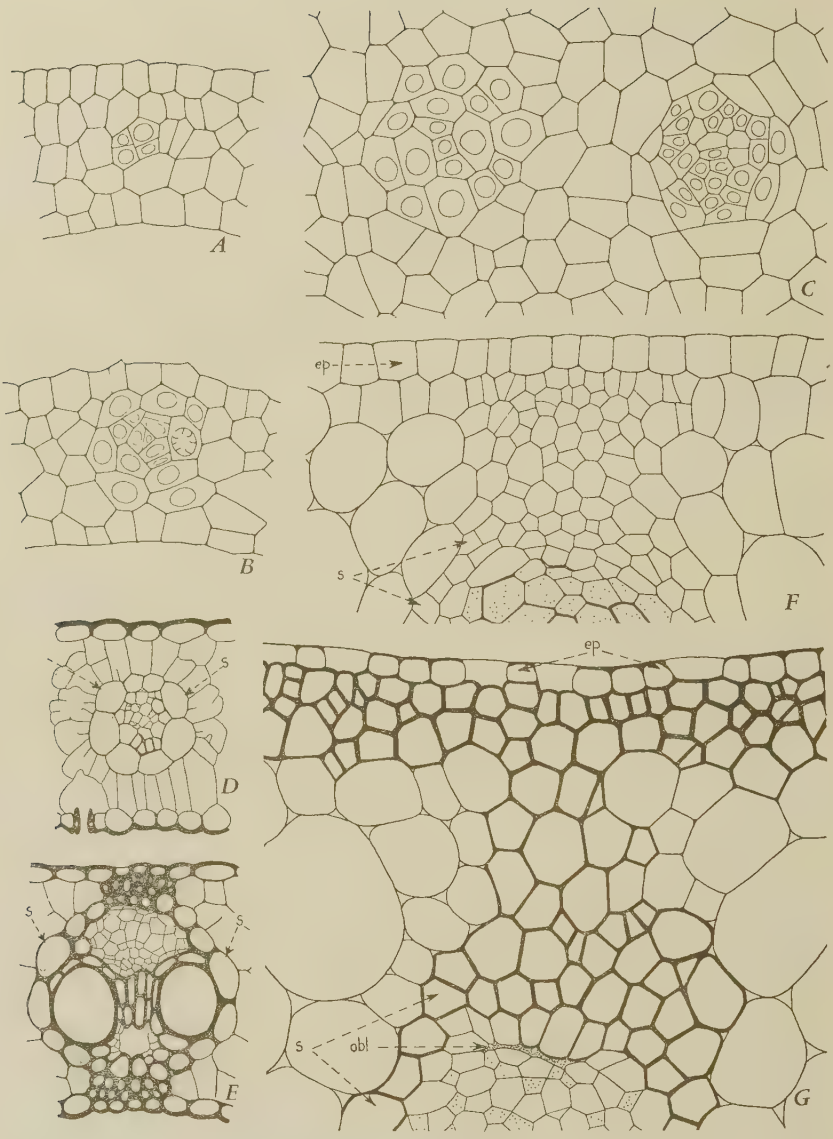

Figure 2.—*A* to *C*, Transverse sections from leaf (*A*, *B*) and stem (*C*) depicting early stages in procambial differentiation. The nuclei visible in these sections are indicated only in the cells that were concerned with the formation of the procambium. *D* and *E*, Transverse sections of mature vascular bundles from a leaf blade. The small cells indicated by stippling are companion cells; the stippled circles represent the chloroplasts. *F* and *G*, Transverse sections of portions of leaves illustrating two stages in the differentiation of the adaxial part of the bundle sheath and of the hypodermal sclerenchyma. *F* was taken from the same section as plate 5, *B*; *G* from the section as in plate 9, *B*. In *F* the entire visible part of the phloem is stippled; in *G* only the companion cells. Details are: *ep*, epidermis; *obl*, obliterated protophloem; *s*, sheath. (*A* to *C*, *F* and *G*, × 467; *D* and *E*, × 160.)

longitudinally and because the ringlike thickening does not occur in this section. The metaphloem shows the usual composition (sieve tubes and companion cells), and the few protophloem elements appear as flattened cells between the metaphloem and the sheath. According to Cheadle (1942), the presence of vessels in leaf bundles is characteristic of the Gramineae.

The smallest bundles have entirely parenchymatous sheaths rich in chloroplasts (fig. 2, *D*). The walls of the sheath cells are somewhat thicker than those of the mesophyll, but are not lignified. According to Strasburger (1891, p. 336), the sheath cells of the small leaf bundles have cutinized radial walls. The xylem and phloem are much reduced in amount as compared with the large leaf bundles. Strasburger describes the tracheary elements as reticulate-pitted vessels (1891, p. 337, "vessel-like tracheids"). The vascular tissues of these bundles mature after the elongation of the leaf and are here interpreted as metaphloem and metaxylem.

The bundles intermediate in size between the largest and the smallest have parenchymatous sheaths. A hypodermal sclerenchyma strand occurs between the epidermis and the sheath—usually on both sides of the bundle, but sometimes on only one side.

The longitudinal strands of the leaf blade are interconnected by transverse anastomoses. In structure these resemble the smallest longitudinal strands. They contain tracheids (Strasburger, 1891, p. 338) and sieve tubes, and are enclosed in a chloroplast-containing parenchyma sheath. According to Strasburger, sieve tubes are absent in the smallest strands. Similar transverse bundles occur in the sheath (fig. 1, *C*, at *a*).

The midvein contains many bundles, the largest resembling the leaf-sheath bundles in size and structure.

ONTOGENY OF THE VASCULAR BUNDLE

Initiation of the Procambium.—The ontogenetic details are discussed with reference to the largest leaf-sheath and stem bundles that appear in mature state, such as the bundle in plate 9, *B*. One may follow conveniently the different stages in the development of the vascular strands by comparing the bundles in leaves of different ages as seen in transverse sections through a shoot apex (fig. 1, *A*, and plate 1).

Sharman (1942) has recently described the origin of leaves and the order of appearance of the procambial strands in *Zea*. Briefly, the leaf formation passes through the following stages: Periclinal divisions in the surface and subsurface cells at the base of the apical cone initiate the leaf buttress. Because of the two-ranked leaf arrangement these

divisions first occur above and opposite the median part of the next lower leaf. From here the divisions spread laterally around the base of the apical cone to form the encircling leaf base. Before completion of this lateral growth, the small protrusion below the apical cone formed by the first divisions begins to grow upward. Thus apical growth and lateral expansion combine to produce a structure that is highest at the point of its origin and that slopes down along the margins, which are in process of encircling the axis. The two margins advancing toward each other first meet (leaf 4 in plate 1), then overlap (leaves 5 to 9 in fig. 1, *A*, and plate 1). In the early stages of leaf development no boundary is evident between the leaf sheath and the lamina (Sharman, 1942). The following discussion of the procambial initiation will simply use, therefore, the word *leaf*. Plate 1 illustrates the nature of these leaves as seen in transverse sections.

Before the primordium completes the encircling of the apical cone and when its median part is about 30 to 40 microns high (this was leaf 1 in the shoot used in plate 1), a central procambial strand appears within the primordium. As the young leaf expands laterally, successive procambial bundles differentiate to the right and left of the median. (Compare the leaves of different age in figure 1 and plate 1.) The median and the first series of laterals are, for convenience, classified here as bundles of the first rank or first order (fig. 1, *D*), since, as a group, they arise first and become the largest in the leaf. Eventually bundles of the second rank are interpolated between those of the first (leaves 7 and 8 in fig. 1, *A*). Then appear bundles of the third rank (leaves 9 to 14 in fig. 1) and those of the fourth (leaf 14 in fig. 1). Still smaller bundles may be formed (Strasburger, 1891). During the formation of the smallest longitudinal strands, transverse anastomoses arise in the leaf (leaf 14 at *a* in fig. 1, *C*). According to Sharman (1942), the median and the large lateral bundles differentiate acropetally within the leaf, whereas the bundles of higher ranks differentiate basipetally and appear at the apex after the laterals reach their highest position. The transverse anastomoses arise after the small longitudinal bundles. These also are formed in basipetal succession (Sharman, 1942). The order of appearance of the smallest bundles is related to the basipetal maturation of the leaves—a characteristic very common among angiosperms.

As shown by transverse and longitudinal sections of the developing leaves, the epidermis is continuous over the leaf margin, and marginal initials add new cells to this tissue layer. Submarginal initials give rise to all the other cell layers of the leaf. Sharman (1942, p. 251) suggests that the dermatogen also may add cells to the inner layers. The earliest (that is, the largest) bundles are initiated when the leaf is five layers

of cells in thickness. The first divisions indicating the beginning of such a bundle occur in the median of the five layers (fig. 2, *A*). First one cell divides; then adjacent cells become involved. The first division of the first cell may be anticlinal, followed by periclinal or oblique divisions of the daughter cells (fig. 2, *A*); or the opposite sequence may occur. Figure 2, *B*, and plate 2, *A*, show the median procambial strand from leaf 2 of the shoot used for plate 1. The two illustrations were taken from two sections 10 microns apart. In the center of the strand is a group of six small cells that resulted from a recent subdivision of three cells. This group could have arisen from one cell. Certain adjacent cells have also divided or were about to divide around the periphery of the group of small cells. Thus the early divisions initiating a procambial strand appear to spread from a center, the latter being formed by the cells that had divided first. The cells around the central group have a tendency to divide and to elongate tangentially with respect to this group (fig. 2, *B*, and plate 2, *A*). The products of these divisions become further subdivided by walls of different orientation, but mostly anticlinal with respect to the periphery of the bundle (plate 2, *B*).

Depending on the size of the bundles, the centrifugal growth of the procambial strands through the addition of cells on its periphery may be smaller or greater. According to Potonié (1886) and the present observations, the extremely small bundles forming the cross connections in the leaves arise entirely within a layer one cell deep and one cell wide.

While the addition of cells occurs on the periphery of the bundle, the cells within it also divide. Since these early divisions are followed by little cell enlargement, the resulting procambial cells are smaller than those from which they arose. Figure 2, *C*, shows graphically the difference in the size of cells at the beginning (bundle to the left) and at a later stage (bundle to the right) of procambial differentiation.

In longitudinal views the procambial cells at a given level of a strand tend to be of the same length, with the transverse walls placed at the same levels. This gives the procambium a storied appearance. The youngest procambial cells are as long as the adjacent parenchyma cells, but rapidly become longer.

After the procambium has thus been organized into strands of narrow elongated cells (plate 2, *B*, bundle to the left), further divisions within the strands increase their thickness in the radial and tangential directions. Since now the cells enlarge somewhat after each division, the size of the procambial cells is not further diminished. When first differentiated the procambium is, as usual, more densely cytoplasmic than the adjacent parenchyma because of the delayed vacuolation in the procambial cells (plate 2, *B*, bundle to the left). After differentia-

tion of the first vascular element (a sieve-tube element) begins, the procambial cells progressively vacuolate. (Compare the successively older bundles in plates 2, *B;* 3, *A;* and 4, *A*.)

The divisions preceding the differentiation of the first sieve tube within the procambial strand are concerned mainly with the radial increase of bundle thickness; that it, they are periclinal with respect to the leaf or stem surface. The cells assume, therefore, a somewhat orderly arrangement, tending toward a radial alignment (plate 2, *B*, bundle to the right; plate 3, *A*). Occasional anticlinal divisions cause the bundles to expand laterally. As the procambial strands increase in circumference, the adjacent parenchyma cells respond by increasing their diameters parallel to the surface of the bundles and by dividing anticlinally to this surface. Occasional periclinal divisions also occur (plates 3, *B;* 4; 5, *A*). Because of these divisions the parenchyma cells around a young bundle appear transitional in size between the cells of this bundle and the parenchyma farther removed.

Since the bundles of different ranks are initiated at successively later stages of leaf development, the later strands appear in more obviously vacuolated leaf sections than the earlier. The bundles of the first rank are formed within the rather meristematic leaf tissue. Leaf 4 in plate 1, for example, shows the first three procambial strands within the still densely cytoplasmic median portion of the leaf. The successive bundles of the first order are initiated closely behind the marginal meristem, as one sees on examining the overlapping meristematic margins of leaves 5 to 7 in plate 1. The smaller strands (bundles of second, third, and fourth orders) are formed by subdivisions of conspicuously vacuolated cells. (Note the high degree of vacuolation of the median portions of leaves 6 and 7, plate 1, in which the bundles of second rank are initiated.) Similarly in the stem, the first and largest traces arise near the apex in a more densely cytoplasmic tissue than the smaller, later bundles. The small horizontal bundles of stem nodes (fig. 1, *D*) and the transverse connections in the leaves appear among the latest in their respective organs, and the vacuolation of the cells that become subdivided in the process of their formation is most conspicuous.

The bundles of successive ranks, within the midrib and sheath, seem to arise successively closer to the abaxial side of the leaf (plate 3, *B*). Actually they all are initiated in the second layer of cells from the abaxial epidermis. The apparent extreme peripheral origin results from the increase in thickness of the median leaf portions through adaxial meristematic activity. Eventually, because of the repeated periclinal divisions, the adaxial periphery of the median part of the leaf appears like a cambium in transverse sections (leaves 6 and 7 in plate 1).

Protophloem and Protoxylem.—As already mentioned, the large bundles are initiated before elongation of the organs in which they occur. Certain phloem and xylem elements (the *protophloem* and the *protoxylem*) mature during this elongation and, as is commonly known, become destroyed before the leaf or axis attains its mature size. Though in the xylem the longitudinal stretching appears to be the main cause of destruction of the conducting elements, the protophloem is obliterated even if longitudinal growth is little pronounced. Thus, according to Strasburger (1891) and the present observations, in the nodes of *Zea* the protoxylem of the large bundle is not converted into a lacuna, but the protophloem is crushed.

The successive stages in protophloem and protoxylem differentiation may be followed in plates 2, *B*, and 3 to 5. Shortly after the procambial strand is delimited, a sieve-tube element is evident near the outer periphery of the strand. In plate 2, *B* (bundle to the right), this sieve-tube element is immature. Though its walls are already somewhat thick and deeply stained, the cytoplasmic contents are still as dense as in the procambial cells. The first sieve tubes in plate 3, *A*, are mature. Their thickened walls and the lack of stainable cell contents make them very conspicuous. Additional protophloem sieve tubes differentiate centripetally from the first, eventually forming a compact cluster of "clear" cells near the outer periphery of the bundle (plate 5, *A*). They are bordered by the young sheath cells on the outside and by the procambium on the inside.

The protophloem sieve tubes lack companion cells. They are enucleate in the mature state and have well-developed sieve plates. Their nacré walls (Esau, 1939), though only moderately thick in paraffin material, are thicker than the procambial walls (plate 5, *A*). Plate 6, *A*, shows parts of the somewhat stretched protophloem sieve-tube elements in a longitudinal view (the two lower cells marked with *st*). The inclined end wall to the right (*sp*) bears a sieve plate, discernible as such under an oil-immersion lens. In this figure the thickness of the walls and the thinness of the protoplasts are obvious. As the protophloem ages and is stretched, the walls become noticeably thinner (plate 5, *B*). Plate 7, *A*, shows part of an old protophloem sieve tube in longitudinal view (the upper cell marked *st*). Since its elements are very long, the transverse walls do not appear in this section; the longitudinal walls are very thin, in sharp contrast to those of the adjacent differentiating metaphloem sieve-tube elements (the lower cell marked *st*).

The destruction of the protophloem is depicted in plates 7, *B*, and 8. The thin-walled protophloem sieve tubes are still intact in plate 7, *B* (two of the outer layers of cells marked *st*). The sheath cells (*s*)

immediately outside the protophloem have divided periclinally. Their subsequent enlargement, combined with the centrifugal growth of the metaphloem, causes a crushing of the protophloem. The first stage in this process is discernible in plate 8, *A;* the second in plate 8, *B*. The final stage of protophloem obliteration appears in plate 9 (at *obl*). In old stem parts the obliterated protophloem shows a strong lignin reaction with phloroglucinol and hydrochloric acid.

When two to three protophloem sieve tubes are mature, the first protoxylem element differentiates. The nature of this and of the subsequent protoxylem elements was determined on the basis of their appearance during development. When the differentiating tracheary elements show secondary thickenings on the longitudinal walls, the entire or most of the transverse end wall—the part which is removed just before maturation—remains free of secondary thickenings and is deeply stained. Since the transverse wall is thicker in the middle, it appears somewhat lenticular in shape in the narrowest elements. Because these developmental stages resemble those observed in the differentiating vessel elements of other plants (Esau, 1936*b;* Esau and Hewitt, 1940), the tracheary protoxylem elements in *Zea* are here interpreted as vessels.

Cheadle (1942) has pointed out that in the mature state the spiral xylem elements of the Gramineae cannot well be studied in macerations; they are too much stretched and too difficult to separate from other cells. Cheadle, however, identified spiral vessels in at least one plant organ in twenty-two species of grasses. In the metaxylem, vessels were found in all organs of forty-five species of thirty-three genera of Gramineae.

The first protoxylem vessel is usually annular and appears near the inner margin of the bundle (plate 4, *A*). Before the first vessel matures, the xylem end of the procambial strand becomes rather more vacuolated than the phloem end (plate 3, *A*). Later the phloem region also vacuolates conspicuously (plate 4). The first and the subsequent protoxylem vessels appear, one after the other, in the same radial row of cells, each successive element being wider than the preceding. (Compare the bundles in plates 4 and 5.) During differentiation of the protoxylem the storied appearance of the procambium is disturbed. While the mother cells of the earlier vessels are elongating, those of the later vessels are still dividing by transverse walls, so that the end walls of the successive vessel mother-cell series occur mostly in different horizontal planes. Plate 6, *A*, shows, above, a portion of a mature annular protoxylem vessel whose segments are longer than the width of the photograph. Then follows a series of vessel mother cells, considerably expanded, but

rather short. Transverse divisions have been completed in this series. The end walls already show the characteristics of vessel end walls before dissolution (Esau, 1936*b*; Esau and Hewitt, 1940). The mother cells of the third series are little expanded and are very short. Obviously this series was still dividing by transverse walls when such divisions ceased in the second series.

The radial alignment of cells in the protoxylem end of the bundle (plates 5, *B*, and 9, *B*) is disturbed by the expansion of the protoxylem vessel segments and by the occurrence of anticlinal and oblique divisions in the adjacent cells. The small cells in this part of the bundle remain parenchymatous (the protoxylem parenchyma).

As previously mentioned, the conducting elements of the protoxylem are destroyed during elongation. When the first vessels are stretched they disappear from view completely, except where a ring happens to occur in a section. Seemingly, in the early stages of this process the parenchyma cells crush the xylem elements. In dicotyledons the xylem parenchyma commonly obliterates the stretched protoxylem cells (Esau, 1936*a*). In *Zea* and other grasses, after several vessels are destroyed, a lacuna is formed because the adjacent parenchyma does not fill the space formerly occupied by the vessels.

The protoxylem vessels mature one after the other and are also stretched successively. Plate 4, *A*, shows a protoxylem vessel before stretching. Every section of the bundle showed a ringlike secondary-wall deposit, because the rings were still close together. In the bundle in plate 4, *B*, the ring appears somewhat tilted. When the rings become widely spaced many sections lack rings (plate 5, earliest xylem elements in both bundles). At this time the younger vessels still have closely arranged rings or spiral coils.

The Metaphloem and Metaxylem.—As shown earlier, the periclinal divisions and the radial seriation of cells resulting from these divisions become evident in the procambial strands before the first conducting elements mature (plate 2, *B*, bundle to the right). This method of cell division continues during the following stages of bundle differentiation. As the cells increase in number, the orderly arrangement of the procambial cells becomes more and more conspicuous (plates 4 and 5). The periclinal divisions occur in several cells of one radial row. In other words, there is no single initial layer like the one commonly thought to occur in the cambium. Nevertheless, in the last stages of cell addition the procambium, as seen in transverse sections, markedly resembles the cambium of the dicotyledons. The dividing cells become limited to a narrow region, and the immediate products of division have short radial diameters (plates 5, *B*; 7, *B*; and 8). The radial

arrangement of cells is later somewhat obscured in metaxylem—as in protoxylem—by enlargement of vessels, though the small-celled part of the metaxylem may remain in orderly arrangement (plate 9, *B*). The mature metaphloem reveals by its pattern the origin from a radially seriated meristem (plate 9).

Plates 7, *B,* and 8 show the differentiation of the metaphloem. The metaphloem sieve tubes have companion cells. As usual, these cells arise from the same phloem mother cells as the sieve-tube elements with which they are associated. A longitudinal division of the phloem mother cell forms a wide and a narrow cell (plate 7, *B,* and 8, *B*). The narrow cell divides again, at right angles to the first plane of division of the phloem mother cell, so that a vertical file of companion cells is formed in connection with each sieve-tube element. The protoplasts of the differentiating companion cells become denser than those of the cells from which they arose (plates 6, *B;* 7, *B;* and 8, *B*).

After the division of the phloem mother cell into the companion cells and the sieve-tube element, the walls of the latter become conspicuously thickened except on the side touching the companion cell (plate 7, *B*). This, the so-called "nacré" wall (Esau, 1939), appears before the nucleus disintegrates (plate 8, *B,* sieve tube with a nucleus, *n,* and a thick wall; plates 6, *A,* and 7, *A*) and shows prominent pit areas (plate 7, *A,* indentations in the thick wall of the lower cell marked *st*). The pit areas become sieve areas (Cheadle and Whitford, 1941); the transverse or slightly oblique end walls differentiate as sieve plates. Plate 9, *A,* shows at the left (above) a mature sieve plate, in which the lighter dots represent the callus cylinders lining the pores. At this magnification the cytoplasmic connecting strands through the callus cylinders are not discernible.

The early metaxylem vessel that occurs to the left of the lacuna in plate 9, *B,* usually begins to differentiate while the organ elongates and shows the effect of this growth in the considerable length of its segments. The two lateral metaxylem vessels are initiated later, are little affected by stretching, and have therefore comparatively short segments. According to Frey-Wyssling (1940), the reduction of the segment length of the last metaxylem vessels, as compared with the earlier vessels, distinguishes *Zea* and other Gramineae from the dicotyledons with secondary growth, in which the metaxylem elements are progressively longer.

The Bundle Sheath.—The foundation of the bundle sheath is laid during the early stages of procambial differentiation. The outermost layer of cells in the bundles of plates 2, *B,* 3, and 4 is a bundle sheath in its earliest stage of development. The young sheath follows the increase

in circumference of the bundle by anticlinal divisions (anticlinal with respect to the periphery of the bundle). Then on the xylem end of the strand the sheath becomes two- to several-layered by periclinal divisions (plates 5, *B*, and 9, *B*). On the flanks also the sheath may be more than one cell thick. In the first stages of bundle development the sheath is uniseriate outside the protophloem (plates 3, *B*, and 4). Later, divisions occur within, and also outside, the original sheath layer (plate 5). The cells resulting from the divisions outside are also added to the sheath.

The amount of sheath tissue added to the bundle in its somewhat advanced stage of development varies with the size of the bundle and its location in the plant. Most massive sheaths develop in the intercalary-meristem region. These are the collenchymatous sheaths (or bundle caps) discussed previously. The compound bundles in this region strikingly illustrate the close developmental relation between the sheaths and the vascular tissues. They become compound through the development of additional small vascular strands within the sheath surrounding a larger bundle. Figure 3 illustrates this phenomenon. The vascular tissues at *a* were the first to differentiate. Cell divisions around this bundle produced the massive sheath. In the outer part of the latter, new vascular groups began to differentiate before the sheath matured into collenchyma. The small strand at *b* had three sieve tubes with companion cells (stippled cells) and a tracheary element, still immature but with secondary walls. In the bundle at *c* two sieve tubes with companion cells were present, and a tracheary element without secondary thickenings. The bundle at *d* showed only an incompletely expanded tracheary element. Although certain cell divisions had occurred around it, no phloem elements had yet differentiated.

The lack of developmental distinction between the sheath and the vascular tissues is revealed also by other bundles. Thus the first xylem element may differentiate from a sister cell of a young sheath cell; and in the small transverse anastomoses the vascular elements and the sheath cells arise from the same parenchyma cell. Then, of course, in the earliest stages of procambial initiation the future vascular cells cannot be separated from the future sheath cells (fig. 2, *A* to *C;* plate 2, *A*).

Similarly, there is no clear demarcation between the sheath and the adjacent tissues on a developmental basis. It has been shown that the procambial strand grows first by the addition of cells on the periphery through division of cells adjacent to the bundle. Often, after the last of these divisions, half of a cell is added to the bundle, whereas the other half remains outside. Then, as already mentioned, new sheath cells may be added to the bundle in the later stages of its development.

The lack of demarcation between the bundle sheath and the adjacent

tissue is most obvious in the bundles that are in contact with the hypodermal sclerenchyma in stem and leaves. This tissue is initiated at the time when the bundle sheath is being thickened by the division of cells located outside the protophloem end of the bundle. The hypodermal sclerenchyma originates by longitudinal divisions of the parenchyma

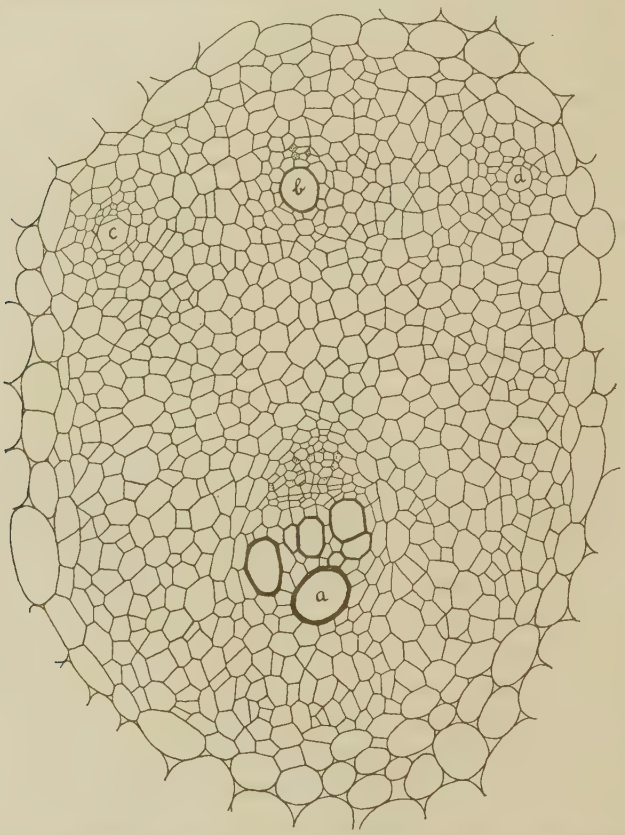

Figure 3.—Transverse section of an immature bundle from the intercalary-meristem region of a leaf sheath. This bundle is a compound structure. Four vascular strands (*a* to *d*) are imbedded in a comparatively small-celled tissue, which at maturity becomes collenchymatous. The vascular strands are in different stages of development, that in *a* being the most advanced and that in *d* the least differentiated. (× 228.)

underlying the young epidermis. In the narrow part of the leaf blade the superficial layer of cells also contributes to this sclerenchyma; in other words, some of the sclerenchyma cells are sister cells of the epidermis. The protodermal origin of peripheral sclerenchyma strands has been previously observed in the monocotyledons (Haberlandt, 1914, p. 203–04). Figure 2, *F*, illustrates an early stage of development of the

sheath and of the hypodermal sclerenchyma in connection with the bundle that appears in its entirety in plate 5, *B*. The epidermis is not involved in the production of this hypodermal sclerenchyma strand. A similar region in a mature state is depicted in figure 2, *G*, prepared from the same section as plate 9, *B*. The sheath and the hypodermal sclerenchyma are connected by a narrow strand composed of sclerenchymatous cells similar to those of the sheath; the hypodermal sclerenchyma has somewhat thicker walls. The epidermal cells adjacent to the hypodermal fibers are flatter than elsewhere (fig. 2, *E*), even if the protoderm does not contribute cells to the sclerenchyma. It appears as though the cell multiplication which occurs during the formation of the bundle cap and the hypodermal sclerenchyma produces a pressure beneath the epidermis so that the cells of the latter do not expand radially. Plate 6, *A*, shows in longitudinal view the region between the protophloem sieve tubes and the epidermis of the leaf containing this protophloem (the upper of the two layers of cells labeled *ep*). The young sheath cells and the differentiating hypodermal sclerenchyma cannot be distinguished from each other.

If the bundle is closer to the periphery of the leaf than the bundles in figure 2, *F* and *G*, the merging of the bundle sheath and the hypodermal sclerenchyma is complete. If the bundle is somewhat farther away, narrow elongated cells with thin cellulose walls intervene between the two tissues. Finally, the hypodermal sclerenchyma may be completely dissociated from the bundle.

The Stage of Bundle Differentiation as Related to the Order of Leaf Origin at the Shoot Apex.—Sharman (1942) has related the anatomical development of *Zea* leaves to the order of their origin at the apex. Since the shoots used in the present study showed a much slower differentiation than the material described by Sharman, the data are presented for comparison.

This paper will not discuss the longitudinal order of differentiation of the vascular system. The writer agrees, tentatively, with Sharman (1942): the median procambial strand and its protophloem differentiate acropetally from the axis into the leaf; and the large lateral bundles are clearly evident when they first appear at the base of the leaf, though they cannot be distinguished at lower levels. Sharman concluded that the lateral strands differentiate basipetally into the axis and acropetally into the leaf from somewhere near the base of the leaf. He also assumes that the median strand has a basipetal course in its lower parts within the stem. His view on the developmental course of the smallest bundles has already been given.

Using as an example the shoot partially depicted in figure 1, the

comparative degree of differentiation of the vascular tissues in the different leaves is as follows:

The shoot was cut consecutively through thirteen internodes. The fifteenth and eighteenth nodes were cut separately. In the latter the long and the short diameters of the axis were 10 mm and 8 mm respectively. Leaves 1 to 6 were 0, 70, 250, 570, 970, and 1,640 microns high, respectively; the node-to-node distances between the nodes 1 and 14 (at these levels the internodes were not differentiated as such or could not be distinguished in cross sections) measured 50, 50, 80, 80, 80, 80, 100, 160, 240, 270, 520, 500, and 900 microns. Leaf 1 was just initiated, and only the median part of its buttress was discernible, whereas leaves 7 to 13 were cut below their apices. In the shoot of plate 1 the first leaf was a few microns high.

The procambial strand of leaf 1 could be discerned only in the axis (fig. 1, *A*). Leaf 2 had the median procambial strand in its base. The median strand of leaf 3 reached to 60 microns below the leaf tip; and the laterals were evident at the base of the leaf, but not in the axis. The traces of leaves 2 and 3 had mature sieve tubes. At a level similar to that in plate 1, the median strand of leaf 4 resembled the bundle shown to the left in plate 2, *B*. Farther below, an immature sieve tube occurred, whereas the trace contained mature phloem elements. The median strand of leaf 5 had reached, at levels above figure 1, *A*, the stage of development depicted in plate 2, *B*, to the right; at lower levels the sieve tube was mature. The median bundle of leaf 6 had two sieve tubes and one xylem element like the bundle in plate 4, *A*, except that the xylem element was not yet mature. It had secondary walls, but the protoplasts were still intact. The secondary walls were evident 1,370 microns below the leaf apex and through several internodes in the axis. On each side of the median bundle were two bundles with one or two mature sieve tubes, but without xylem. Still farther toward the leaf margins followed three or four bundles with immature sieve tubes; then several procambial strands without vascular elements. Divisions to form bundles of second rank occurred in leaf 6 (plate 4, *A*, below).

Leaves 1 to 6, available in their entirety, had not yet ceased their apical growth, as was evidenced by the highly meristematic appearance of their apices. The marginal growth was still evident in leaf 11. These data strikingly contrast with those of Sharman (1942), in whose material plastochrone 6 was characterized by a fully expanded lamina with metaxylem and metaphloem forming basipetally in the sheath undergoing its final elongation, whereas the marginal meristems were becoming inactive in plastochrone 4.

The median strand of leaf 7 (the first that was cut below its apex)

of the present study had reached the stage of development depicted in plate 3, *B*, to the left. The xylem element was somewhat stretched in the median levels, but not above and below. On one side of the median strand were two bundles with two sieve tubes and one vessel each, the latter not yet stretched. Then followed a strand with an immature tracheary element and some sieve tubes. The others had no xylem. In about three more bundles, mature sieve tubes were present. On the other side of the median the bundle development reached almost the same stage, except that there was still no mature xylem.

The median strand of leaf 8 was somewhat younger than the bundle in plate 5, *A*. The first protoxylem vessel was noticeably stretched in the median levels available, but not in the upper sections and not at the insertion of the leaf. Bundles of the third rank in their initial stages of development occurred in the sections of leaf 8. The median of leaf 9 was somewhat larger than that of 8, having three mature protoxylem elements. At higher levels the first element had almost disappeared because of stretching and crushing, whereas the second was somewhat stretched. At the base of the leaf, which was noticeably more meristematic than the upper regions, the first element, though slightly stretched, was apparently still intact. The first xylem in the laterals was also somewhat stretched above.

Though the stretching of the first two protoxylem vessels was very pronounced in the median bundle of leaf 10 at higher levels, very little of it occurred below. In leaf 11 the fourth protoxylem vessel was differentiating acropetally; in leaf 12, the fifth. Both these leaves indicated considerable stretching of the first vessels at their bases. The median strands of leaves 10 to 12 showed the successive early stages of metaphloem and metaxylem differentiation (plates 5, *B*, and 7, *B*). This process was taking place in the basipetal direction, as indicated by the younger appearance of the bundles below. Particularly striking was the disappearance, toward the leaf bases, of the expanding lateral metaxylem vessels. Among the basipetally differentiating elements was also a centrally located vessel, which Sharman (1942) apparently called the last protoxylem. As was mentioned previously, judging by the time of its development it might also be called metaxylem. This central vessel had secondary walls and intact protoplasts above in the median strand of leaf 13. Farther down, the secondary walls were absent; and at the base of the leaf all evidences of metaxylem differentiation had disappeared. Of the five acropetal protoxylem vessels previously formed in the median strand of leaf 13, four were much stretched above and were associated with a lacuna. In fact, the number of vessels involved in the formation of the lacuna could not be established at the upper

levels, so pronounced was the rupture of the first three. At the base of the leaf, however, all five vessels were intact, though the first two were much stretched. The first bundles of the second rank had mature sieve tubes in leaf 11; those of the third rank in leaf 13.

The central basipetal vessel was mature above in leaf 14; farther down, the protoplasts were intact; and 650 microns above the union of the leaf with the stem the secondary walls had disappeared. A prominent

TABLE 1

LENGTHS IN CENTIMETERS OF THE ASSOCIATED LEAF BLADES, LEAF SHEATHS, AND INTERNODES OF A VIGOROUSLY GROWING ZEA SHOOT; AND CERTAIN BUNDLE CHARACTERISTICS OF THE INTERNODES*

Leaf and internode number†	Leaf blade	Leaf sheath	Internode	Certain bundle characteristics of the internodes
1	67	1.2	0.4	First protoxylem elements mature
2	80	2.0	0.8	No protoxylem lacuna
3	100	4.0	1.0	Lacuna barely indicated
4	110	....	2.0	Metaphloem and metaxylem just initiated
5	110	....	5.0	.....
6	120	22.0	14.0	Lacuna formed, protophloem crushed, lateral metaxylem vessels still with nuclei
7	120	24.0	22.0	Metaxylem mature, sheath immature
8	...	....	21.0	Fully mature bundles
9	...	....	15.0	Fully mature bundles
10	...	....	6.0	Fully mature bundles

* Data adapted from Strasburger, 1891.
† Leaves and internodes are numbered consecutively beginning with the youngest; no. 10 is the lowermost on the stem.

lacuna appeared in the place of the first four vessels at higher levels. The fifth vessel was intact. The basipetal central vessel occurred in the same radial row as the acropetal vessels and was in lateral contact with the fifth protoxylem vessel. Below, near the leaf insertion, at least three of the acropetal protoxylem elements were intact. Here the lacuna was absent. The expansion of the central and of the lateral metaxylem vessels had progressed to the base of the leaf.

At higher levels of leaf 15 all the bundles were mature, those of the first rank appearing like the bundle in plate 9, *B*. Toward the base, however, part of the metaphloem was still immature, the lateral metaxylem vessels had no secondary thickenings, and the sheath had thin, nonlignified walls. The lowest part of leaf 15 was not available, but the next larger leaf showed at least three intact protoxylem vessels in the median bundle. Finally, leaf 18 showed at the very base the stages of final development of bundles characteristic of the "adult" intercalary meristem. One of the smaller lateral bundles from this region appears in figure 3. The collenchyma is not yet thickened, and the metaxylem not

yet fully mature. The median bundle showed two intact protoxylem elements, secondary walls and protoplasts in the metaxylem vessels. The metaphloem was almost mature.

As Sharman (1942) has explained, the basipetal maturation of the metaxylem and metaphloem within the leaf is related to the long-delayed intercalary elongation of the leaf sheaths. The relatively slow development of the sheaths is evident from Sharman's (1942, p. 248) table 1 and from Strasburger's (1891, p. 357–61) data. The latter are summarized as table 1 in the present paper. The internodes attain their mature length still later than the sheaths; and the maturation of the vascular bundles in the internodes occurs, as in the sheaths, after their elongation (column 4 in table 1; also Sharman, 1942).

The picture obtained from all these data is as follows: Before and during the elongation of the shoot, the protoxylem and protophloem form connections between the growing organs and the mature parts of the plant. After the elongation, the metaxylem and metaphloem are formed. These tissues mature first in the leaf blade, because it attains its mature length first; then their differentiation progresses through the leaf sheath into the internode, maturing in these organs after they complete their intercalary growth. The bundle sheath matures in the same direction. The special characteristics of the bundle sheath and metaxylem of the intercalary zones of the leaf sheaths and internodes make possible a further, probably limited elongation of these organs.

The continuity of the vascular tissues across the growing regions is maintained, presumably, through the relative structural plasticity and developmental adjustments of the protophloem and protoxylem. The elements of these tissues are capable of some extension in the mature state without, apparently, being destroyed. With further stretching they are destroyed, but the protoxylem (and protophloem) elements differentiate one after the other and are destroyed in a similar succession. Such method of growth would seem to provide some intact elements during the entire period of elongation, until afterwards the metaxylem and metaphloem mature in the completed internode and sheath.

Sharman (1942, p. 274) assumes, however, that when the protoxylem is ruptured in the elongating organs the leaf has no direct connections with the stem by means of differentiated water-conducting cells and that therefore the water must move part of its way across living cells. Sharman's own data do not prove this point. One might surmise that in table II (Sharman, 1942, p. 268) leaf 6 illustrating the basipetal differentiation of the metaxylem is left without xylem elements between levels 34 and 37. His text figure 21 (p. 270), however, indicates the presence of intact protoxylem at both these levels. The condition of the

xylem in the remaining 4 cm of this leaf is not mentioned. Similarly, in all other illustrations, immature metaxylem is associated with some intact protoxylem in the same bundle.

In the present writer's experience, eosin solution passes readily through the elongating internodes and leaf sheaths of *Zea* and other grasses, just as it would pass through open water-conducting channels. Under the dissecting microscope the stained bundles appear entirely continuous and (in materal stained with phloroglucinol and hydrochloric acid) show some mature water-conducting elements in sections taken at different levels; metaxylem elements at the higher, more mature regions of the leaf; and protoxylem elements in the growing zones. The data obtained from the paraffin sections, given earlier in this paper, also indicate continuity of the conducting channels across the active intercalary meristems. Further studies using a wide range of material would be desirable to test the value of Sharman's assumption.

DISCUSSION

In the present paper the terms *protophloem* and *metaphloem, protoxylem,* and *metaxylem* are used in the sense in which they were originally conceived and in keeping with the writer's views as previously expressed (Esau, 1943). In this classification of the primary vascular tissues the relation of their development to that of the organ or plant as a whole is more important than their morphological characteristics. Thus employed, the concepts of *protophloem* and *metaphloem, protoxylem* and *metaxylem* serve the distinctly useful purpose of giving a dynamic picture of plant structure and of clarifying the relation between the development of the plant and the function of the vascular system. The protophloem and the protoxylem are quickly initiated in the shoot apex and (through their relative structural and developmental plasticity) maintain the continuity of the conductive channels in the growing regions. The less plastic but more elaborate metaphloem and metaxylem assume the function after the plant organs reach their mature size. Though the statement above consists of well-known facts, the repetition seems justified in view of various recent attempts to reevaluate the categories of the primary vascular tissues, to give them new meanings, or even to discard the classification entirely—all on the basis of very limited morphological or developmental facts. (See review by Esau, 1943.)

Incidentally, the vascular bundles of *Zea* (and probably of other Gramineae) offer excellent class material to show the student the distinctive features of the successive parts of the primary phloem and primary xylem. In contrast to Stover's (1934) results, the present study

demonstrates a rather clear distinction between the protophloem and metaphloem of *Zea*.

The present investigation bears upon the problem of the developmental relation between the bundle sheaths and the vascular tissues of the Gramineae. These sheaths obviously develop as integral parts of the vascular bundle. Similarly related to the vascular tissues from the developmental standpoint are all the different types of sheaths in *Zea* —that is, the parenchymatous sheaths of the small bundles within the leaf blade, the sclerenchymatous sheaths of the large and small bundles in the other parts of the plant, and finally the collenchymatous sheaths in the intercalary region. The compound bundles of the intercalary zone show in a most striking manner the close relation between the sheath and the vascular tissues: additional vascular strands develop within the bundle cap of an earlier, larger strand. Strasburger's (1891, p. 345) classification of all bundle sheaths in *Zea* as parts of the stele ("Stelolemmae") seems practical.

This classification involves, however, some difficulties. The bundle sheaths, particularly the collenchymatous and the sclerenchymatous, cannot be sharply separated, on the basis of origin, from the adjacent parenchyma and from the sclerenchyma strands that do not constitute parts of the bundles. The similar origin and development of the intra- and extra-fascicular sclerenchyma illustrate Foster's (1942, p. 32) statement that "the nomenclature and classification of cell types and 'tissues' is still in a confused and uncertain state." Classifying the fibers, Foster (1942, p. 74) follows Haberlandt (1914, p. 152–55) in placing all the extracambial fibers into the group of "bast" fibers. The sclerenchyma of the monocotyledons also belongs in this category. As Foster states, this is a "topographical" classification; it is doubtless the most convenient at present. Haberlandt (1914, p. 199–202) goes even further in identifying the extra- and intrafascicular fibers by interpreting the meristem giving rise to the extrafascicular fibers as *procambium*. Plate 6, *A*, in the present paper shows how such a concept could be formulated: the procambium between the xylem and phloem displays the same morphology as the meristem between the protophloem and the epidermis that forms the bundle sheath and the extrafascicular hypodermal sclerenchyma. Further comparative developmental studies are needed before Haberlandt's use of the term *procambium* can be properly evaluated.

The procambium that gives rise to the vascular bundles in *Zea* shows a predominance of tangential divisions, a tendency most pronounced in the late stages of bundle development. This method of division causes a resemblance between the procambium and the cambium of the dicoty-

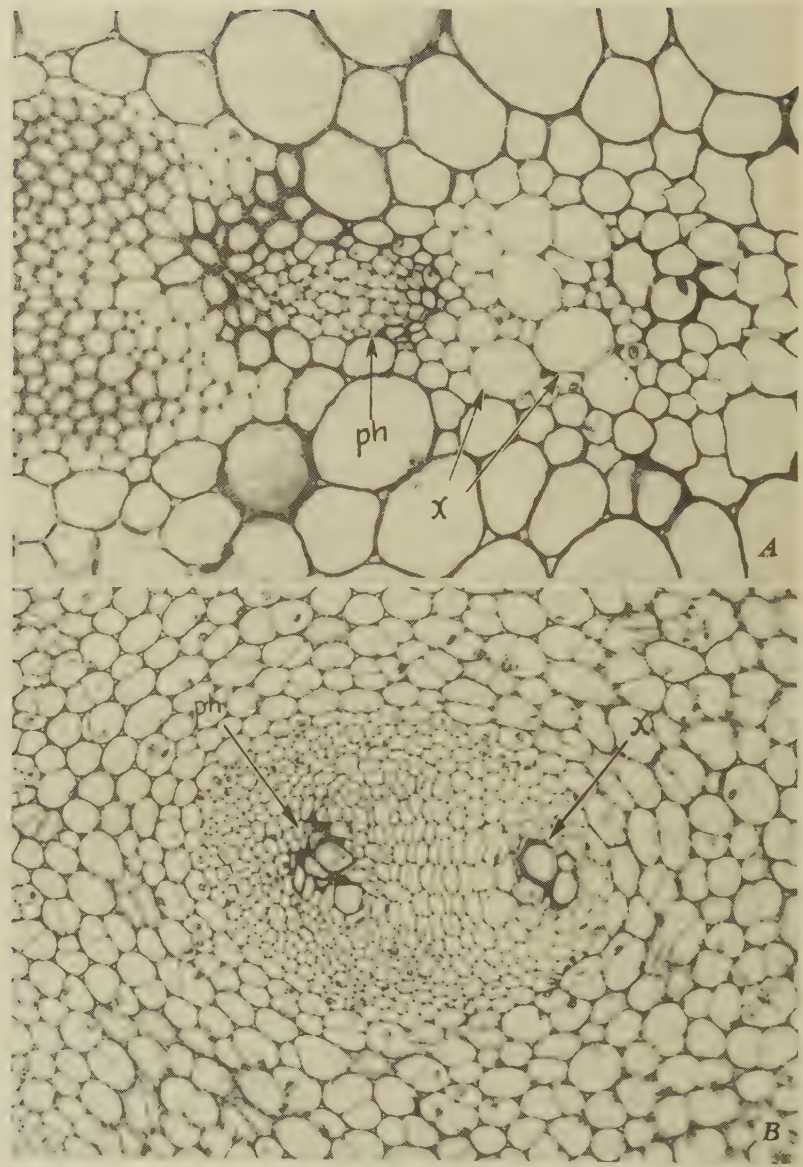

Figure 4.—*A*, Transverse section of a vascular bundle from a leaf of *Iris germanica*, showing the orderly radial seriation of the mature metaphloem (*ph*). The xylem (*x*) appears to the right of the phloem. *B*, Transverse section of a vascular bundle from a young leaf of *Cocos nucifera*, illustrating the cambiumlike appearance of the meristem that gives rise to the metaphloem and metaxylem. The protophloem (*ph*) and the protoxylem (*x*) are differentiated in this bundle. (Both × 250.) (From slides lent by Dr. V. I. Cheadle.)

ledons as seen in transverse sections. The radial seriation of the procambium cells is very common in the monocotyledons. Thus figure 4, *B*, shows an immature bundle of a *Cocos* leaf, with orderly aligned meristematic cells between the protoxylem and the protophloem. The literature contains many more examples. (See review by Esau, 1943.) Sometimes the radial seriation is clearly maintained by the mature tissues, as in the instance of the metaphloem of *Iris* in figure 4, *A*. As previously emphasized (Esau, 1943), the problem of tissue classification has been only confused by the attempts of many workers (recently Sharman, 1942) to use the radial seriation of the meristematic cells as the principal criterion for distinguishing between the procambium and the cambium. A broad flexible classification, taking into consideration developmental aspects of the plant as a whole, has already been advocated by the writer in reference to the vascular meristems, as well as the vascular tissues (Esau, 1943). All the vascular tissues of *Zea* are here interpreted as primary and arising from procambium. These vascular tissues are a part of the wholly primary plant body. In certain arborescent monocotyledons the primary body is amplified by the addition of secondary tissues (mainly vascular).

Recently Moreland and Flint (1942, p. 361) interpreted the cross connections in the sheath of the sugar cane as secondary because "they arose from living cells that had become permanent and then returned to the meristematic condition." Modern morphologists of course regard living parenchyma cells (or, indeed, any living cell) not as "permanent," but as potentially meristematic (Hayward, 1938, p. 14; Bloch, 1941; Foster, 1942, Exercise III and p. 58; and others). Almost obsolete, therefore, is the distinction of the secondary tissues from the primary on the basis that the former arise from cells that returned to the meristematic condition. The present study, furthermore, by comparing the development of the cross connections with that of the other bundles, has revealed a whole series of transitions from the bundles of the first rank (formed within the still rather densely cytoplasmic cells) to the smallest bundles that arise in highly vacuolated parenchyma. The cross connections are parts of the primary vascular systems of the wholly primary plant bodies of corn and sugar cane.

SUMMARY

The procambial strands of *Zea mays* are initiated through longitudinal divisions in localized areas of the differentiating organs. The procambium of the largest strands is formed first in the still comparatively dense cytoplasmic parts of the shoot. The smaller bundles arise later in the more highly vacuolated part. A series of transitions occur

between the largest and the smallest bundles with regard to relative time of appearance in the differentiating organs and also with regard to the degree of vacuolation of the parenchyma from which the procambium arises.

More cells subdivide in the formation of the larger strand than in the development of the smaller ones. In its origin the procambium is not sharply separated from the adjacent parenchyma : when cells divide to produce procambium, part of a cell may become added to the procambium, while the other part may remain outside.

After the procambial strand is delimited, it increases in thickness by divisions within it. Radial growth dominates over the tangential; that is, tangential divisions predominate. As the bundle advances in its development, this orientation of the planes of division becomes increasingly evident. Despite the cambiumlike appearance of the vascular meristem, it is here interpreted as procambium, and all the vascular bundles in the whole plant are considered to be primary.

The first protophloem sieve tubes mature before the first protoxylem vessels. The protophloem is composed of sieve tubes only. In this it contrasts with the metaphloem, in which companion cells are associated with the sieve tubes. The protophloem is gradually crushed while the metaphloem matures. In like manner the conducting elements of the protoxylem are gradually destroyed while the metaxylem matures.

The bundle sheath differentiates partly from the outermost layer of the procambium strand, partly by addition of cells from the adjacent parenchyma, which undergo longitudinal divisions. Thus the bundle sheath is a part of the vascular bundle, but is not sharply separated from the tissue outside the bundle. The bundle sheath may be confluent with the hypodermal sclerenchyma, which arises partly like the peripheral sheath cells, by divisions and elongation of parenchyma cells, partly from the derivatives of the protoderm.

The protophloem and protoxylem are the conducting tissues of the elongating part of the shoot. The maturation of the metaphloem and metaxylem is delayed until the organs complete their elongation.

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PLATES

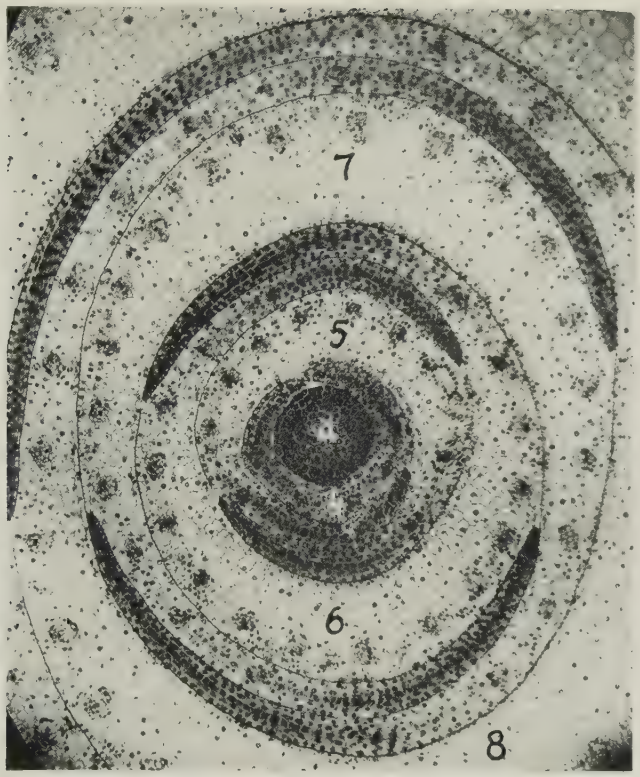

Plate 1.—Transverse section through the apex of a shoot, showing the stem apex (*a*) and the leaves enclosing the stem. The youngest leaf visible in this section was the fourth from the apex. Therefore the leaves in this figure are numbered 4 to 8. The three younger leaves occurred in sections cut at lower levels of the shoot. (× 77.)

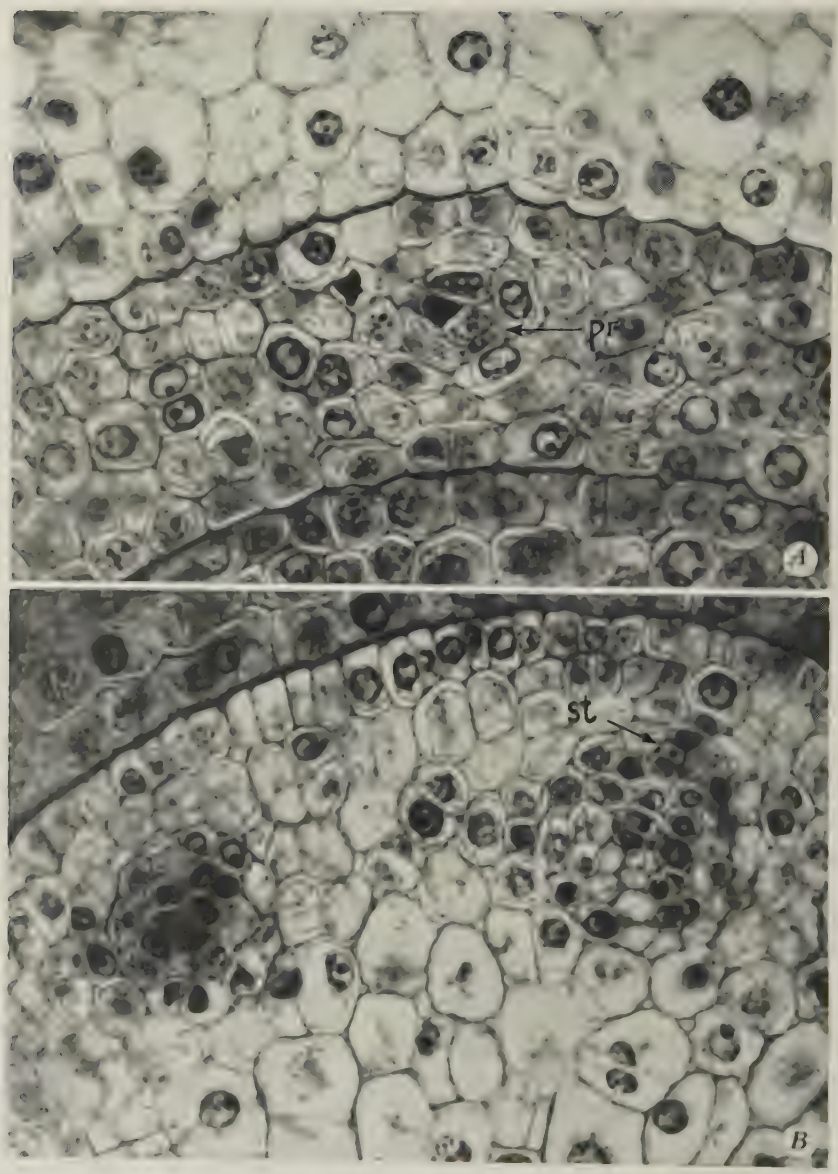

Plate 2.—Transverse sections of parts of leaves, showing early stages in the differentiation of the procambium. In *A* adjacent the very young median procambial strand (*pr*) of the second leaf below the apex. *B* shows the median (right) and the adjacent lateral (left) bundles of the fifth leaf below the apex (leaf 6 in plate 1). The median bundle has an immature sieve tube (*st*). (Both × 750.)

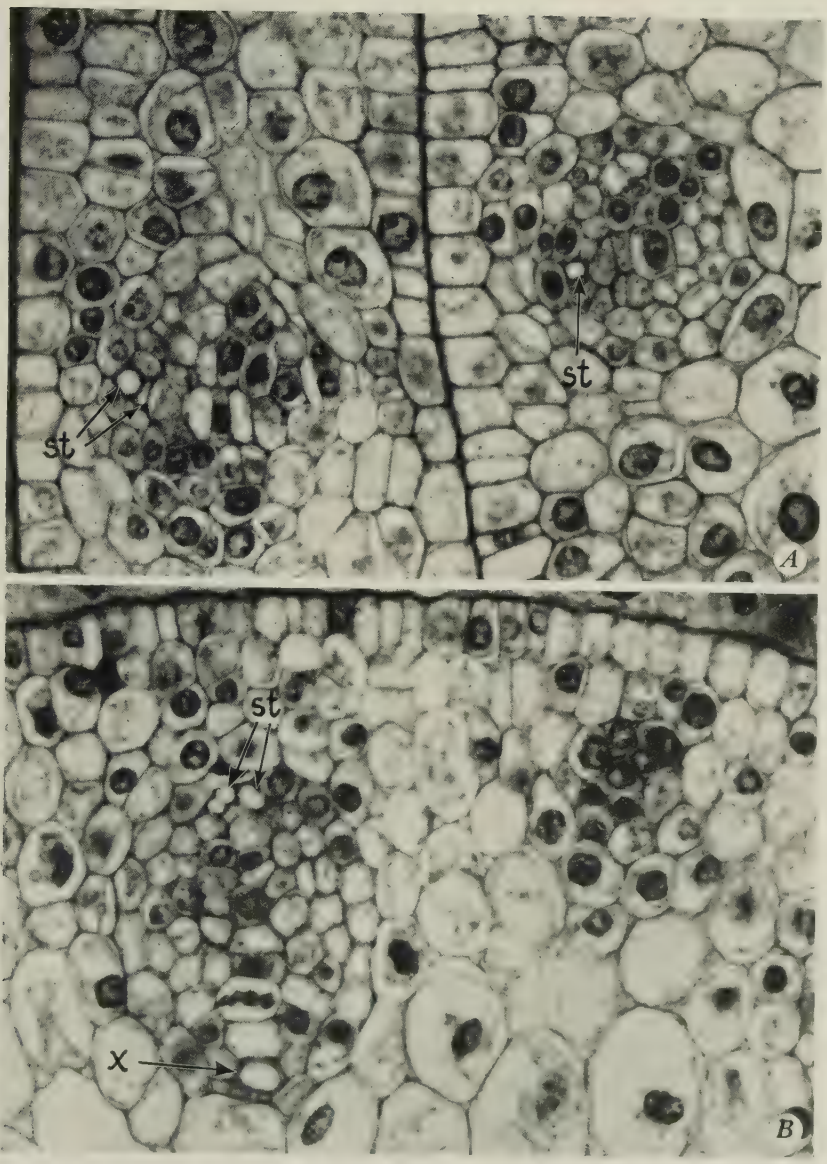

Plate 3.—Transverse sections of parts of leaves, showing early stages of vascular differentiation in the procambial bundles. In *A* is illustrated two first-rank lateral bundles, one (right) from the seventh, the other (left) from the eighth leaves below the apex. In each bundle the first sieve tube (*st*) is mature. In the bundle to the left the second sieve tube is almost mature. At *B* is shown, to the left, the median strand of leaf 7 with three mature sieve tubes (*st*) and one mature xylem element (*x*). A small procambial strand of second rank (bundle II in fig. 1, *D*) appears to the right in *B*. (Both × 750.)

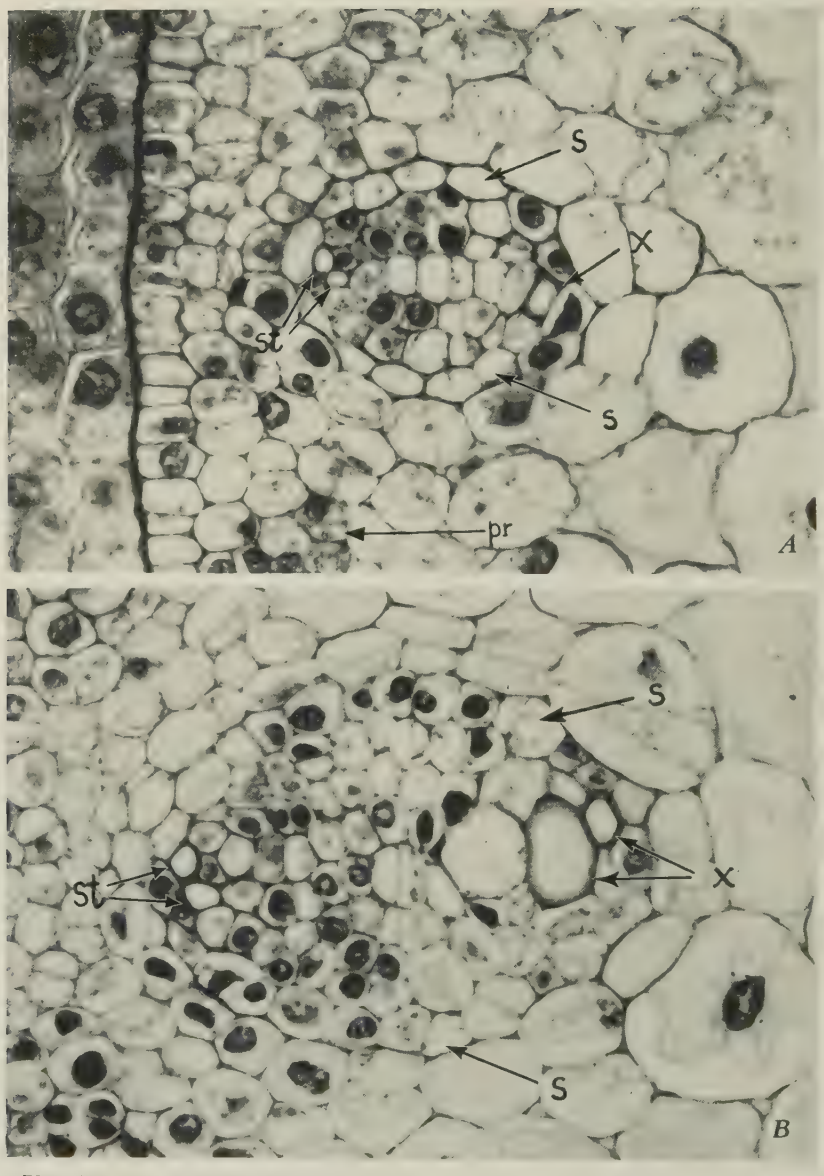

Plate 4.—Transverse sections of bundles from the sixth (*A*) and eighth (*B*) leaves below the apex. These were bundles of the first rank (bundles I in figure 1, *D*) located next to the median strands. The bundle in *A* shows two sieve tubes and two xylem elements. The smaller of the two xylem elements in *B* has been stretched somewhat, and the ringlike secondary wall thickening appears slightly tilted. Details are: *pr*, procambium; *s*, sheath; *st*, sieve tube; *x*, xylem element. (Both × 750.)

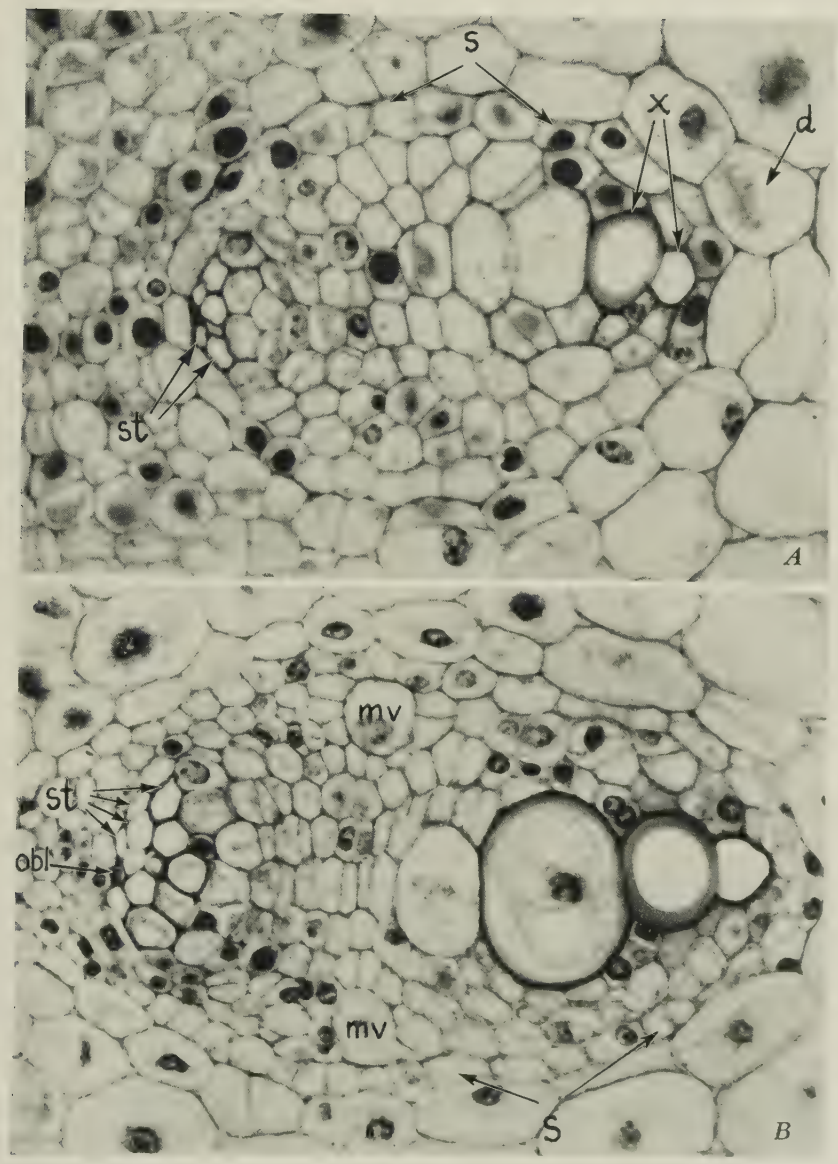

Plate 5.—Transverse sections of bundles showing an earlier (*A*) and later (*B*) stages in the differentiation of the protophloem and protoxylem. *B* shows also the early stage of metaphloem and metaxylem development. The bundle in *A* occurred in the tenth, the bundle in *B* in the thirteenth leaves below the apex. Both were lateral bundles of the first rank. Details are: *d*, dividing cell; *mv*, lateral metaxylem vessels; *s*, bundle sheath; *st*, sieve tube; *x*, xylem element. (*A*, × 750; *B*, × 480.)

Plate 6.—Longitudinal sections of a younger (*A*) and an older (*B*) vascular bundle. The bundle in *A* was somewhat older than the bundle in plate 5, *A*; the bundle in *B* was comparable with the one in plate 9, *A*. Details are: *c*, companion cell; *ep*, epidermis (the two adjacent layers of epidermis belong to two different leaves); *mv*, metaxylem vessel element; *s*, sheath; *sp*, sieve plate; *st*, sieve tube; *v*, vessel or vessel element. (*A*, × 400; *B*, × 290.)

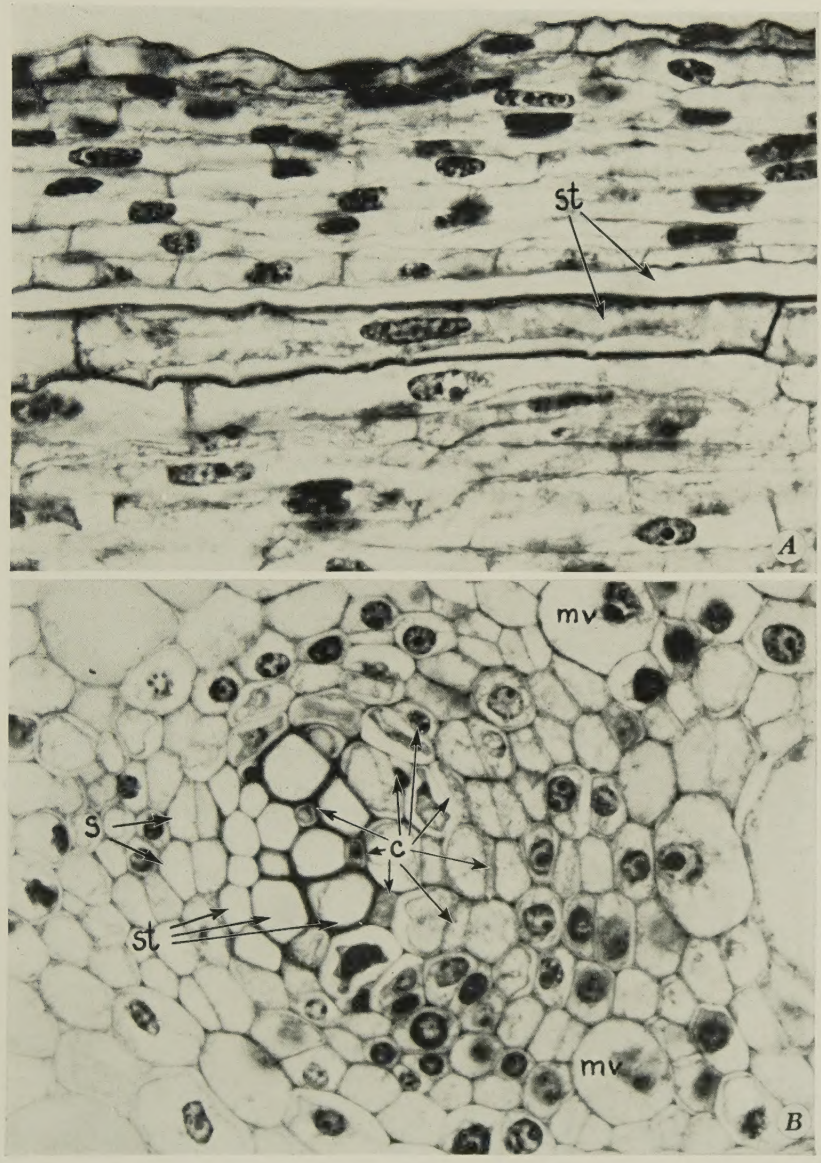

Plate 7.—*A*, Longitudinal section of a vascular bundle, showing a differentiating metaphloem sieve-tube element (lower cell labeled *st*) with pitted nacré walls and a portion of an old and much elongated protophloem sieve tube (upper cell labeled *st*). *B*, Transverse section of part of a bundle showing mature protophloem (thin-walled cells apparently without contents to the left in the bundle). The first sieve tubes of the metaphloem with nacré walls and companion cells occur to the right of the protophloem. The sheath cells (*s*) outside the protophloem have divided by periclinal walls. Details are: *c*, companion cell; *mv*, metaxylem vessel; *s*, sheath; *st*, sieve tube. (Both × 750.)

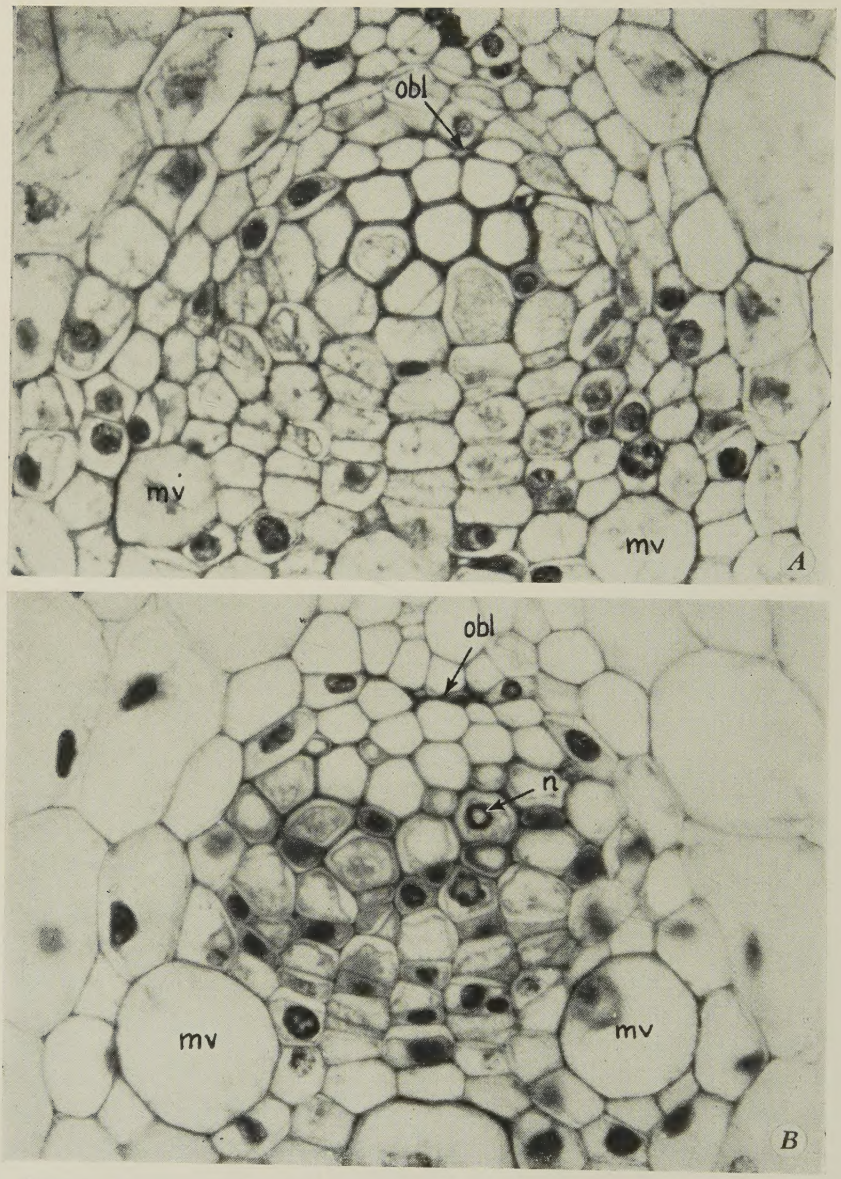

Plate 8.—Transverse sections of parts of bundles showing two stages in phloem differentiation. In *A* is illustrated the beginning of protophloem crushing at *obl* and an early stage in metaphloem development. At *B* is depicted the stage of active metaphloem differentiation. Details are: *mv*, metaxylem vessel; *n*, nucleus; *obl*, obliterated protophloem. (Both × 750.)

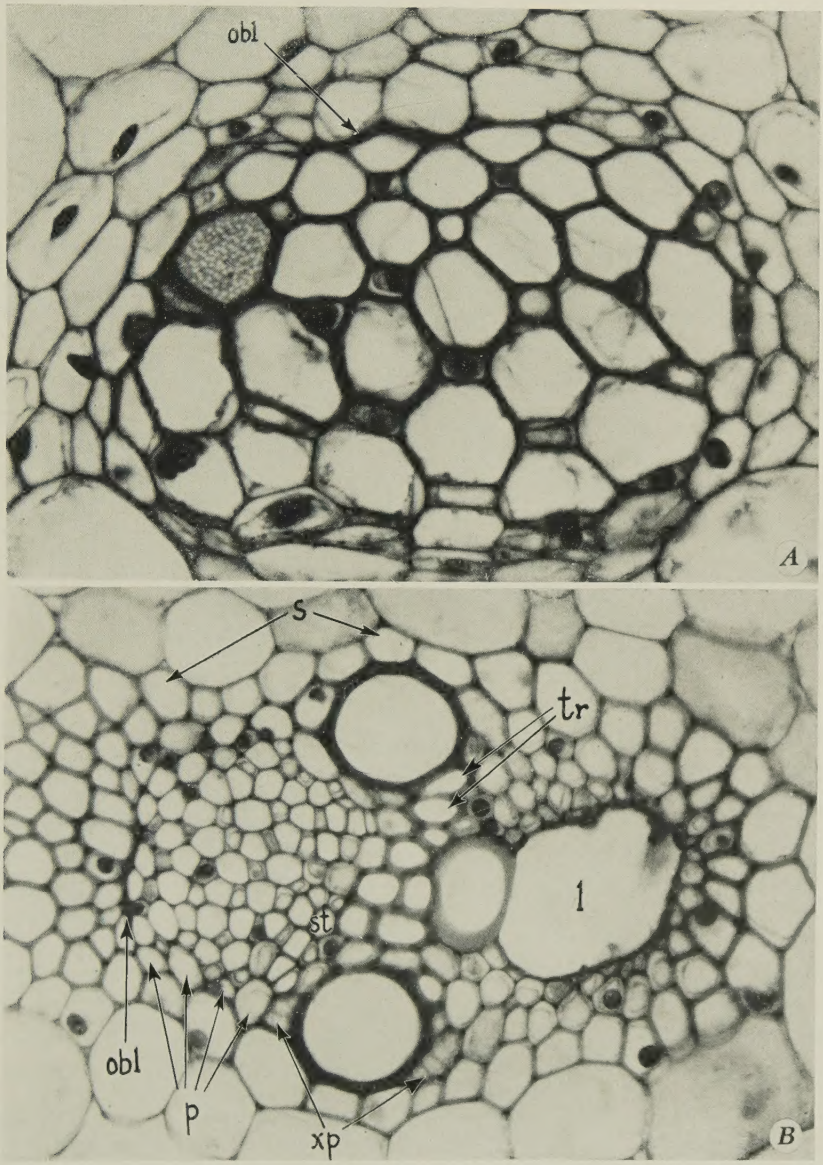

Plate 9.—*A*, Transverse section of almost mature metaphloem from a bundle in an internode. The protophloem is obliterated (*obl*). The first metaphloem sieve tubes are somewhat smaller than the later. A sieve plate is visible in one of the sieve tubes to the left. *B*, A mature vascular bundle from leaf 15 in transverse section. Details are: *l*, lacuna of the protoxylem; *obl*, obliterated protophloem; *p*, parenchyma; *s*, sheath; *st*, sieve tube; *tr*, tracheary element; *xp*, xylem parenchyma. (*A*, × 750; *B*, × 480.)

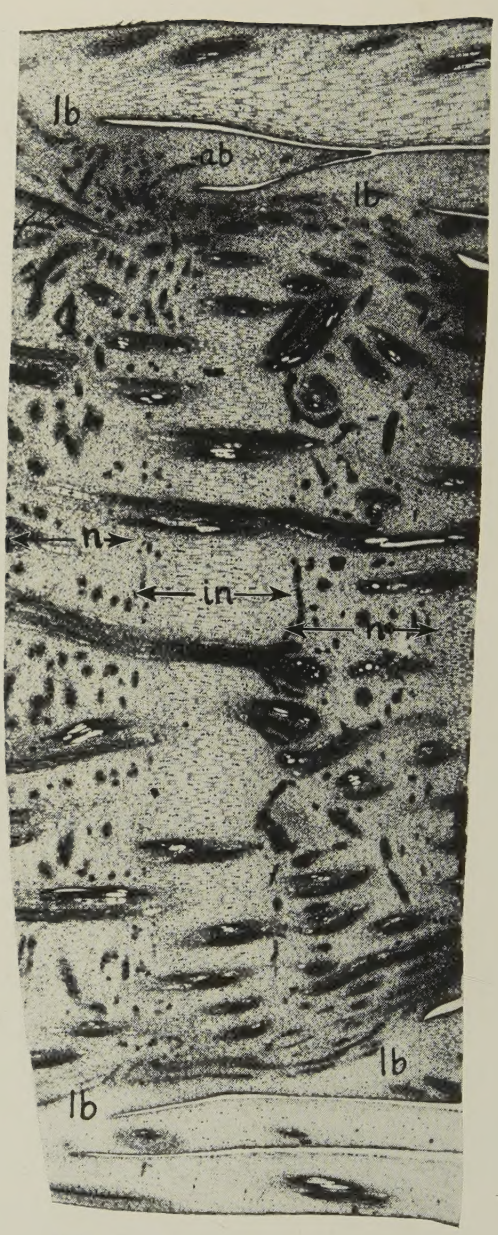

Plate 10.—Longitudinal section through two nodes (*n*) and an internode (*in*). The leaf bases (*lb*) attached to these nodes are approximately the sixteenth and seventeenth from the apex. A part of an axillary bud appears at *ab*. The figure illustrates the continuity of the vascular bundles from node to node across the elongating internode. (× 20.)